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CONTENTS OF VOLUME II

SEPTEMBER, 1915. NUMBER 1

	PAGE
ON THE OCCURRENCE OF A TRYPAÑOPLASM, PROBABLY <i>Trypanoplasma borreli</i> LAVERAN ET MESNIL, IN THE BLOOD OF THE COMMON SUCKER, <i>Catostomus</i> <i>commersonii</i> . J. W. MAVOR.....	1
(With one plate)	
TWO NEW CASES OF POLYRADIATE CESTODES, WITH A SUMMARY OF THE CASES ALREADY KNOWN. WINTHROP D. FOSTER.....	7
SOME NOTES AND EXPERIMENTS ON <i>Sarcocystis tenella</i> RAILLIET. JOHN W. SCOTT	20
EGG VARIATION IN A TREMATODE SPECIES. WILLIAM WALTER CORT.....	25
SOME NEW GEGARINÆ PARASITES FROM ARTHROPODA. MINNIE ELIZABETH WATSON	27
(With two plates)	
<i>Pneumonyssus foxi</i> nov. sp., AN ARACHNOID PARASITIC IN THE LUNG OF A MONKEY (<i>Macacus rhesus</i>). FRED D. WEIDMAN.....	37
(With one plate)	
CESTODE CYSTS FROM MUSKRAT. EDWIN LINTON.....	46
SARCOPHAGID LARVAE FROM THE PAINTED TURTLE. F. E. CHIDESTER.....	48
NOTES	50

DECEMBER, 1915. NUMBER 2

PROFESSOR VON PROWAZEK.....	51
(With portrait)	
FURTHER NOTE UPON COMPARISON OF <i>Endamoeba gingivalis</i> (GROS) AND <i>Endamoeba histolytica</i> SCHAUDINN. ALLEN J. SMITH AND M. T. BARRETT	54
NOTES ON THE TREMATODE GENUS TELORCHIS WITH DESCRIPTIONS OF NEW SPECIES. HORACE W. STUNKARD.....	57
(With one plate)	
THE INSECT VECTOR OF UTA, A PERUVIAN DISEASE. CHARLES H. T. TOWNSEND	67
<i>Filaria cingula</i> PARASITIC IN THE SKIN OF <i>Cryptobranchus allegheñiensis</i> . FREDERIC H. KRECKER.....	74
THE LIFE HISTORY OF <i>Gongylonema scutatum</i> . BRAYTON H. RANSOM AND MAURICE C. HALL.....	80
NOTE ON THE STAGE OF <i>Piroplasma bigeminum</i> WHICH OCCURS IN THE CATTLE TICK, <i>Margaropus annulatus</i> . HOWARD CRAWLEY.....	87
THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON, PROCEEDINGS.....	93
NOTES	96

MARCH, 1916. NUMBER 3

	PAGE
LA FAMILLE DES THELAZIIDAE. A. RAILLIET.....	99
SEASONAL DISTRIBUTION OF SOME ACANTHOCEPHALA FROM FRESH-WATER HOSTS. H. J. VAN CLEAVE.....	106
ON THE INTERMEDIATE HOSTS OF THE LUNG DISTOME, <i>P. westermanii</i> KERBERT. SADA O YOSHIDA.....	111
(With one plate)	
GONGYLONEMA IN THE RÔLE OF A HUMAN PARASITE. HENRY B. WARD....	119
(With one plate)	
ARE SARCOSPORIDIA ABERRANT FORMS OF CNIDOSPORIDIA OF INVERTEBRATES? B. GALLI-VALERIO	126
THREE NEW GREGARINES FROM MARINE CRUSTACEA. MINNIE E. WATSON..	129
(With one plate)	
THE PAJAROELLO TICK (<i>Ornithodorus coriaceus</i> KOCH) WITH SPECIAL REF- ERENCE TO LIFE HISTORY AND BITING HABITS. WILLIAM B. HERMS....	137
NOTE ON THE ETIOLOGY OF VERRUGA AS DEDUCED FROM A STUDY OF THE ASEXUAL STAGES OF BARTONELLA. CHARLES H. T. TOWNSEND.....	143
A NEW INFUSORIAN PARASITE IN SAND FLEAS. MINNIE E. WATSON.....	145
REVIEWS	147

JUNE, 1916. NUMBER 4

THE SIGNIFICANCE OF CERTAIN NATURAL FLAGELLATES OF INSECTS IN THE EVOLUTION OF DISEASE IN VERTEBRATES. H. B. FANTHAM AND ANNIE PORTER	149
A REVISION OF THE GENUS ARHYTHMORHYNCHUS. H. J. VAN CLEAVE.....	167
(With two plates)	
SOME NOTES ON THE ENCYSTED LARVA OF THE LUNG DISTOME. SADA O YOSHIDA	175
<i>Cylindrotaenia americana</i> NOV. SPEC. FROM THE CRICKET FROG. MINNIE E. JEWELL	181
(With one plate)	
THE EFFECT OF TICK BITES ON MAN. D. McCaffrey.....	193
THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON, PROCEEDINGS.....	195
REVIEWS AND NOTES.....	201

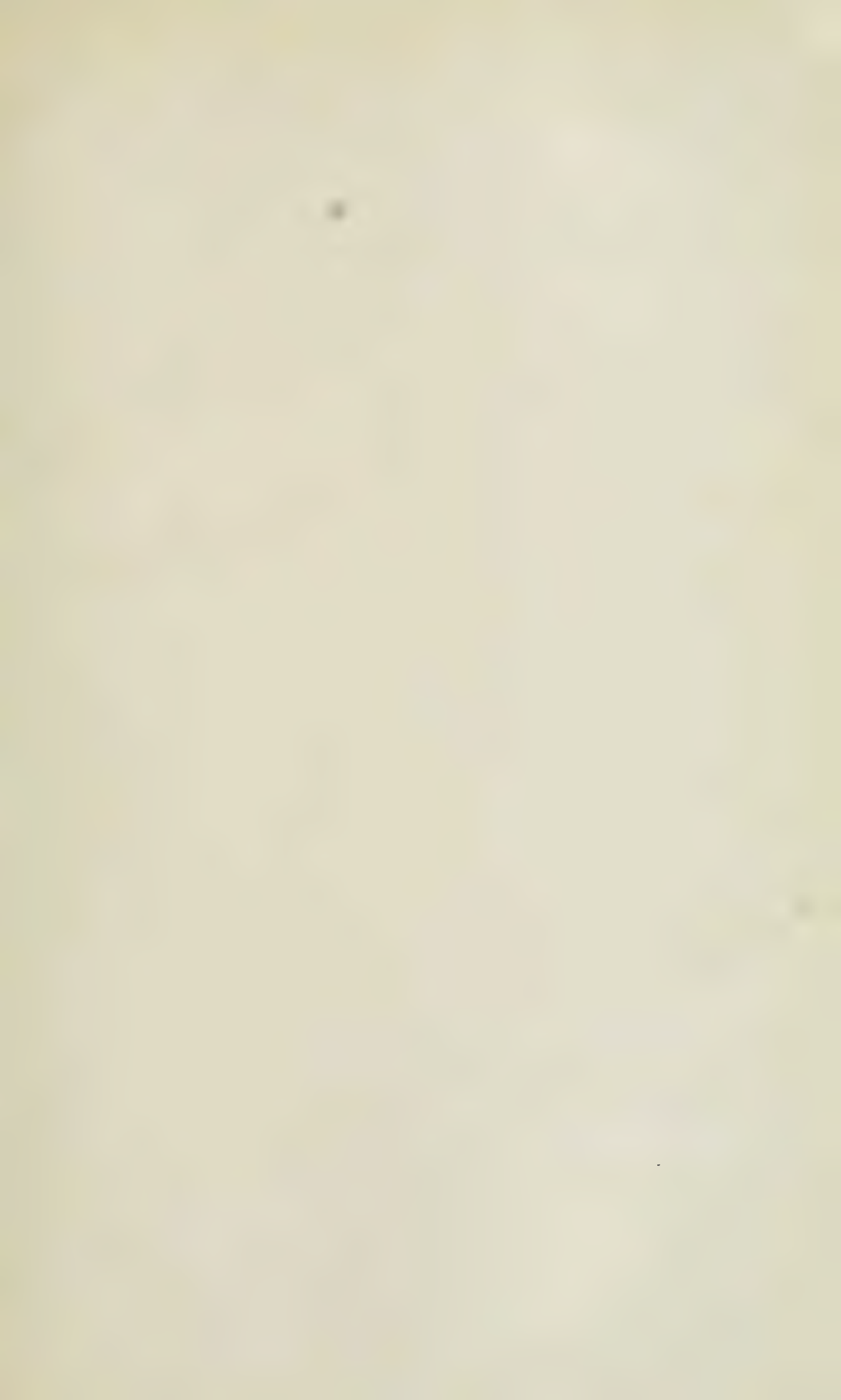
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EXPLANATION OF PLATE

Trypanoplasma, probably *T. borreli*. All the figures with the exception of Figure 1, which is a free-hand drawing, were drawn with the camera lucida, using a Leitz 2 mm. apochromatic objective and compensating ocular $\times 18$. They have been reproduced at a magnification of 2,600 diameters. Figures 2-6 were drawn from smears fixed with osmic acid vapor, stained with Giemsa's azur-eosin and mounted in neutral Canada balsam.

Fig. 1. Drawn from a living specimen in a fresh preparation of the blood.

Fig. 2. From blood from the kidney.

Fig. 3. From blood from the heart.

Figs. 4 to 6. From blood from the kidney.

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Volume 2

SEPTEMBER, 1915

Number 1

ON THE OCCURRENCE OF A TRYPANOPLOASM, PROBABLY *TRYPAÑOPLASMA BORRELI* LAVERAN ET MESNIL, IN THE BLOOD OF THE COMMON SUCKER, *CATOSTOMUS COMMERSONII*¹

J. W. MAVOR

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In spite of the considerable interest in the distribution of the hemoflagellates, there is no record, so far as the writer is aware, of a trypanoplasma occurring in the New World. The species to be described seems to be identical with *Trypanoplasma borreli* described by Laveran and Mesnil (1902). This species is reported to cause disease and death in fish in captivity, but no case is recorded of a trypanoplasma causing a pathogenic condition in a fish in the wild state, where there is little opportunity for more than a single infection. On this account the facts to be recorded are of special interest.

The sucker in which the trypanoplasma was found was seen in shallow water at a wharf in Go Home Bay, a small bay leading from the Georgian Bay, about twenty miles from Penetanguishene, Ontario. The fish was sluggish and allowed itself to be easily picked up in a dip net. When brought to the laboratory and taken out of the water it died in a few minutes. There were no external lesions and no abnormalities were discovered in a hasty examination of the viscera. The gills were pale and bloodless. Preparations of blood from the heart showed the presence in great abundance, one or two in a single field of the immersion objective, of the trypanoplasma to be described.

As the Biological Station was about to be closed for the season it was possible to obtain only one other sucker, which was caught in a fyke net. This was active and normal in every way. In five fresh preparations of the blood from the gills, the heart, and the liver no hemoflagellates were found after a careful search.

The evidence available in this case is scarcely sufficient to prove the trypanoplasma as a specific cause of the pathogenic condition of the

1. The observations in this paper were made while the writer was curator of the Biological Station of the Canadian government at Go Home Bay, near Penetanguishene, Ontario, in 1913. The courtesy of the directors of the Biological Board of Canada permits their publication here.

fish. The parasite is interesting, however, in that it occurs in a fish in the wild state, where there is little probability of there being more than a single, or at most, a few infections, the parasite being without doubt carried by a leech. There is no record, so far as the writer is aware, of a trypanoplasma causing a pathogenic condition in a fish in the wild state. Such a condition, however, has been recorded for fish in captivity, and if, as the author is inclined to believe, the parasite of the sucker is *T. borreli*, for the same species of parasite. In carp, *Cyprinus carpio* L., infected with *T. borreli*, Marianne Plehn (1904:175) finds: "Bei stark befallenen Fischen erreicht die Anämie einen ganz extremen Grad; man kann nur wenige Tropfen eines wässerigen, kaum rötlichen Blutes gewinnen; Keimen und innere Organe sind äusserst blass. Andere pathologisch-anatomische Merkmale fehlen. Die Tiere zeigen in der letzten Lebenszeit ausser beschleunigter Atmung und grosser Unlust sich zu bewegen nichts Auffälliges. Sie gehen offenbar an Blutmangel ein, den sie, zwar lange, aber doch nicht dauernd ertragen können. Es ist unzweifelhaft, dass die Krankheit auch im Freien Schaden anrichten wird; die Beobachtungen sind noch zu jungen Datums um allgemeine Angaben über Verbreitung und Bedeutung zu gestatten." Keysselitz (1906) has also described the pathological condition in the carp due to *T. borreli*. Leger (1904:824) describes cases of acute infection of the minnow, *Phoxinus phoxinus* Agass., with a trypanoplasma as follows: "Des infections aussi intenses amènent chez le poisson une anémie profonde: décoloré et enflé il se tient immobile, refuse toute nourriture et finit par mourir." A case in which a trypanosome may cause a pathogenic condition has been recorded by Doflein. He remarks (1909:398), referring to *Trypanosoma carassii*: "Ich selbst hatte allerdings einmal Gelegenheit, eine sehr ähnliche, vielleicht sogar identische Art im Blut der Schleie, *Tinca vulgaris*, zu beobachten; die befallenen Schleien waren offenbar krank, sie waren sehr apathisch und waren an die Station zur Untersuchung von Fishkrankheiten in folge eines grossen Sterbens in den betreffenden Weihern eingesandt worden."

The terminology used in this paper is that used by Minchin (1912), with the exception of the term "basal granule," which has been used in place of the term "blepharoplast," as used by that author.

As seen in the living state (Fig. 1), the trypanoplasma has the form typical of the genus; a thick yielding body with two flagella, one of which forms the border of an undulating membrane. The measurements of the body are, length 20-25 μ , thickness 3-4 μ . The anterior flagellum is between one-half and two-thirds as long as the body. The posterior flagellum arises near the anterior flagellum and passes posteriorly, forming the margin of the undulating membrane. It extends

freely for about two-fifths of its length beyond the posterior end of the body. The undulating membrane is comparatively thick and not sharply distinguishable from the edge of the body. The parasites showed an active writhing motion, but little progression. What there was seemed to be with the morphologically posterior end in advance. The protoplasm was finely granular, a few larger highly refractive greenish granules measuring up to $0.4\ \mu$ in their longest diameter, being present in the posterior half (Fig. 1). After the preparation had been standing for a little time, sealed with vaselin, two or three large vacuoles were seen in the posterior end of some individuals.

Smears of the blood from the heart and from the kidney were fixed in the vapor of osmic acid, stained with Giemsa's azur-eosin and mounted in Canada balsam neutralized with lithium carbonate. In each of the smears the trypanoplasma was found to be abundant. Although often much distorted, the parasite is found in some parts of the smears remarkably well preserved. It is to be regretted that when the parasite was discovered time did not permit of the making of "wet" smears stained with hematoxylin. The results especially as regards the kinetonucleus are open to criticism on that account.

About fifty of the best-preserved and most clearly showing individuals have been studied in detail with a 2 mm. apochromatic objective and compensating oculars 12 and 18. The parasites show great uniformity in size and general structure. They are nearly always sickle-shaped, the trophonucleus and the undulating membrane being on the convex side. Measurements show little or no difference in size between fresh and preserved individuals.

The protoplasm, as in the fresh preparations, is finely granular and contains a varying number of relatively large, deeply staining granules distributed either mainly in the posterior end (Fig. 3), or irregularly throughout its extent (Figs. 4, 5). It is possible that these larger granules are identical with the greenish granules observed in fresh preparations. Such "chromatoid granules" have been found in different species of trypanoplasms (Leger, 1904; Keysselitz, 1906; Friedrich, 1909; Minchin, 1909). It is doubtful whether these granules are chromidial in nature.

The anterior flagellum arises at the extreme anterior end and on the side of the body on which the kinetonucleus is located. It leaves the body independently of the posterior flagellum (Figs. 4, 5). The posterior flagellum arises very close to the anterior and passes around the blunt anterior end and along the entire length of the body as the margin of the undulating membrane (Figs. 1, 2). It is continued posteriorly as a free flagellum of a length equal to about two-thirds that of the body. The undulating membrane shows in some cases as

a clear unstained area between the posterior flagellum and the granular protoplasm of the body (Figs. 2, 4).

The kinetonucleus (Woodcock, 1909, for the "Geisselkern" of German authors) is situated on the side opposite to the trophonucleus and the undulating membrane and about one-third of the length of the body from the anterior end. It has a clear outline and stains deep purple, in contrast to the more reddish tinge of the trophonucleus. In the individuals which show least distortion it is ovoid, about half again as long as it is wide and shows a distinct membrane. Its size varies between wide limits (Figs. 2-5). Its usual size, however, is 3.5 by 2.4μ . When not too deeply stained five or six deeply staining bodies can be seen lying immediately under the membrane. In some cases what appears to be two kinetonuclei are present in individuals which show no division of the flagella or trophonucleus (Figs. 3, 5). In such cases each of the two bodies shows a clear contour and is undoubtedly surrounded by a membrane. Each also shows the included stained granules, as in the case of the single kinetonucleus, but the number of granules in each is less than in the single kinetonucleus. The two bodies may be of almost equal size, or one, always the anterior, may be much the smaller (Figs. 3, 5); they may be near together or far apart.

That this dual nature of the kinetonucleus is due to faulty technic seems hardly possible, in view of the fact that the two parts are surrounded by distinct membranes. It may be that it is due to division, the kinetonucleus, in this case, having completely divided before either the flagella or trophonucleus. Against this assumption are the facts: first, that the individuals show no other signs of division, unless, which is doubtful, the presence of two basal granules is to be taken as such; and second, that the two parts of the kinetonucleus are often of very unequal size.

Keysselitz (1906) finds in *Trypanoplasma borreli* Laveran et Mesnil that the "blepharoplast" (kinetonucleus) divides transversely, but says (p. 32): "Ein zeitlich gesetzmässiges Verhalten zwischen der Teilung des Kernes und Blepharoplasten kann ich nicht konstatieren." Friedrich (1909:385) finds that the division of the kinetonucleus is "eine einfache Längsspaltung." His figures 19 and 22 show that it may divide before either the trophonucleus or the flagella. Martin (1910) finds in *Trypanoplasma congeri* Elmhirst and Martin that the divisions of the flagella and the trophonucleus precede that of the kinetonucleus.

The condition found in the blood of *Catostomus commersonii* seems to resemble most closely that described by Keysselitz (1906:25) for *Trypanoplasma borreli* in "geschwächten anämischen Fischen" (see his Figs. 40, 42, 45i). Here, however, the kinetonucleus may be divided

into more than two parts. In this connection it will be remembered that the sucker in which the trypanoplasma was found showed an anemic condition similar to that described by Keysselitz. The same author (1906:37 and Fig. 47) finds in a parasite of the stomach and adjacent parts of the alimentary tract, "Bei *Trypanoplasma ventriculi* weist der Blepharoplast sehr häufig eine Sonderung in zwei Stücke auf." Laveran and Mesnil (1902) in their description of *T. borreli* figure two individuals (p. 491, Figs. 13 and 15), each with two kinetonuclei. (Although Laveran and Mesnil considered these bodies to represent the trophonucleus "le noyau" and not the "centrome des Trypanosoma," there is no doubt that they were mistaken.)

Two basal granules (centrioles, blepharoplasts of Minchin, 1912) are usually to be seen where the flagella arise. They stain deeply and are to be distinguished only by their position from the chromatoid granules found in other parts of the protoplasm. Although the ends of the flagella can in certain animals be seen to enter the protoplasm separately (Figs. 4, 5, 6), they cannot be traced to separate granules (Figs. 4, 5).

The trophonucleus is situated about a third of the length of the animal from the anterior and often lies side by side with the kinetonucleus (Figs. 2, 3, 5); in other cases it is slightly behind the kinetonucleus (Figs. 4, 6). Its shape and size resemble that of the kinetonucleus, being however usually slightly smaller. It is ovoid and measures on the average 2 by 3 μ . In many cases it shows a distinct membrane (Figs. 2, 4). In other cases such a membrane was not to be seen, probably on account of poor fixation. It contains a varying number of deeply staining granules. In some cases one of these granules (karyosome?) is larger and centrally located.

So far as the writer is aware the genus *Trypanoplasma* has been described only from European fishes. The species recorded as occurring in the blood are:

T. abramidis Brumpt 1906.

T. barbi Brumpt 1906.

T. borreli Laveran et Mesnil 1902.

T. cyprini Plehn 1904.

T. guernei Brumpt 1906.

T. gurneyorum Minchin 1909.

T. keysselitzi Minchin 1909.

T. truttae Brumpt 1906.

T. varium Leger 1904.^a

The species *abramidis*, *barbi*, *cyprini*, *guernei*, *gurneyorum* and *truttae*, as described by their authors, have a rod shaped kinetonucleus and the free portion of the posterior flagellum either half as long

(barbi) or less than half as long as the anterior flagellum; two characters which exclude the *Trypanoplasma* of the sucker. The latter differs, also, from *T. keysseltzi* which has the two nuclei near together at the anterior end and the kinetonucleus "very elongated". There seems some doubt as to whether *T. varium* is not the same species as *T. borreli*, the chief argument of Leger (1904^a) being that the two forms show a preference for different hosts.

The trypanoplasm found in the sucker has all the morphological characters described and figured for *T. Borreli* by Laveran and Mesnil (1902), size and shape of the body, position and shape of the nuclei, and length of the flagella. The writer therefore provisionally identifies it with this species.

It is interesting to note that German carp, in which Keysseltz (1906) has studied *T. borreli*, have been introduced into the Canadian lakes and occur near where the sucker was found. *Catostomus commersonii* and *Cyprinus carpio* are closely related fish, being in the same family, Cyprinidae.

It is therefore not improbable that *T. borreli* has been introduced into the Canadian lakes with the German carp.

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TWO NEW CASES OF POLYRADIATE CESTODES, WITH A SUMMARY OF THE CASES ALREADY KNOWN

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Anomalies in adult cestodes are by no means uncommon and have been reported by numerous investigators in the last two centuries. These may be divided into those which affect only part of the worm and those which are characteristic of the entire strobila. Among the former are supernumerary proglottids, fenestrated segments, bifurcation of the strobila at the posterior end, branching at the side, forming a short chain of proglottids arising from a supernumerary proglottid, fusion of the line of separation of the proglottids through part of the strobila, and inversion of the usual arrangement of the sexual organs. Supernumerary proglottids are usually formed by the insertion of a smaller incompletely developed proglottid, into an otherwise normal proglottid (Fig. 1) or the supernumerary proglottid may be triangular and extend the entire length of the lateral edge of the proglottid to which it is attached. Fenestrated proglottids in which the central portion of the parenchyma is lacking are usually confined to a few segments, but in a few cases have involved nearly all the segments of the strobila. Fusion may be complete throughout a large number of proglottids or the line of demarcation between the proglottids may be obliterated only partially, giving the appearance of an excessively long proglottid on one side and two or more normal proglottids on the other, as in McCulloch's (1913) case.

Of more immediate interest are the cases of triradiate strobilae, in which the entire strobila instead of being flat or ribbon like, as in the normal tapeworm, has a central ridge extending uniformly throughout all the proglottids and giving a triradiate figure when seen in cross-section. This anomaly may also be combined with the anomalies affecting individual proglottids mentioned in the preceding paragraph. Twenty-eight such cases have been collected by Vigener (1903) and are summarized in the accompanying table. To this list I have added five more cases, one of them not hitherto reported.

EXTERNAL ANATOMY OF POLYRADIATE CESTODES

Analogous to the triradiate forms are those in which the tapeworm has more than three wings. Such forms are far less common, and if we except Leuckart's case (1880), which he considered as a fusion

of two triradiate forms, but which Barrois (1893) concludes is a case of simple triradiate proglottids having a simple supernumerary proglottid attached to one of the wings, only one case (Rosenberger, 1903) has hitherto been reported. In Rosenberger's case the parasite was pentaradiate, forming a star-shaped figure. As Rosenberger, however, does not specify the exact shape of the proglottids other than to describe them as "star shaped," and as his observation was published in a journal not readily accessible to European helminthologists, it has been largely overlooked and the existence of strobilae having more than three wings is not mentioned in any of the text-books. In the present paper a tetraradiate proglottid of *Taenia saginata* is described. The term *polyradiate* is suggested as a convenient word for describing all cases of adult cestodes in which the strobila is formed of three or more wings radiating from a common axis.

With but one exception, all triradiate cestodes in which the head has been recovered have been found to have six suckers instead of the usual four; the triradiate feature extending throughout the scolex, as is well seen in Vigener's (1903) case. In Rudolphi's (1810) case, however, the scolex is described as normal. Considering that the principal feature of this anomaly is its uniformity throughout the entire strobila, and that Rudolphi's case is the only exception recorded, the correctness of his observation has been questioned. It is therefore logical to assume that a cysticercus with six suckers represents the larval stage of a triradiate cestode, and this view is generally accepted by helminthologists. Such cases are included in Vigener's (1903) list of triradiate cestodes on which the present table is based.¹ In all, forty-four cases of polyradiate cestodes (larvae and adults) have been reported. The number of individual specimens reported is, however, much greater, since two writers, Zürn (1898) and Railliet (1899), report seeing "several" larval cestodes having six suckers, and three other writers each describe two or more adult specimens.

By far the greater number of polyradiate forms are found in *Taenia saginata*, twenty-four adult cestodes of this form having been described. Of these twenty-four cases, two (Andry, 1741, and Brera, 1811) are so indefinite as to be doubtful, and in four other cases the distinction between *Taenia solium* and *Taenia saginata* has not been made. These cases are assigned to *T. saginata*, since this parasite is more common than *T. solium* in the regions where the cases were observed. Among the twenty-four cases is one pentaradiate form (Rosenberger, 1903) and one tetraradiate (Foster, the present paper).

1. Vigener's article includes a complete bibliography of all cases of triradiate cestodes then known.

In four species, *Taenia saginata*, *T. solium*, *T. pisiformis*, and *T. coenurus*, larval forms with six suckers have been found as well as the adult triradiate forms. Summarizing the cases the number of individual specimens reported are:

Adult cestodes		Larval cestodes	
Species	Number	Species	Number
<i>Anoplocephala perfoliata</i>	1	<i>Coenurus cerebralis</i>	2
<i>Bothriocephalus latus</i>	1	<i>Coenurus serialis</i>	Several
<i>Bothriocephalus tectus</i>	Several	<i>Cysticercus bovis</i>	1
<i>Dipylidium caninum</i>	1	<i>Cysticercus cellulosae</i>	2
<i>Taenia coenurus</i>	3	<i>Cysticercus pisiformis</i>	1
<i>Taenia echinococcus</i>	1	<i>Cysticercus tenuicollis</i>	Several
<i>Taenia pisiformis</i>	1		
<i>Taenia saginata</i>	24		
<i>Taenia solium</i>	2		
<i>Taenia taeniaeformis</i>	2		

It appears from the column in the table showing the localities where polyradiate cestodes have been found that this anomaly is as widespread as the geographical distribution of the cestodes themselves. That more cases have been reported from Germany and France than elsewhere, is apparently due to the greater attention which has been given to the subject of teratological forms in these countries.

In most cases of adult polyradiate cestodes the scolex has not been recovered. Twenty-four writers have recorded cases of *Taenia saginata*, but five of them only have observed the head. This is probably due to the fact that these specimens were recovered from the living hosts by vermifuges instead of at autopsy, and were therefore more liable to damage. The fact that cases of polyradiate cestodes have been found far more commonly in *Taenia saginata* than in other species may be due to a greater frequency of variation characteristic of this species, as suggested by some helminthologists, but may also be explained by the fact that this species, being a common parasite of man, is perhaps more frequently observed than any other species. This opinion is supported by the fact that most of the cases are reported by practicing physicians who have many opportunities to observe this species and little chance to study other species not parasites of man. If this species were especially subject to this anomaly we should expect to find a correspondingly large number of *Cysticercus bovis* with six suckers, yet only one such case has been reported.

A triradiate cestode usually has an unpaired wing, smaller than the other two wings, which are usually of nearly equal size. Sometimes these equal wings lie close together, as in MacCallum's (1912) case, giving the worm the appearance of a normal cestode split lengthwise through half its width. Usually, however, the wings are thickened at the base so that they are separated from one another, giving a triradiate appearance. The unpaired wing may be so reduced as to form a mere ridge along the center of an otherwise normal parasite, as in

Jelden's (1900) case, or it may be so well developed as to be equal in size and symmetry with the other wings, giving a perfectly symmetrical figure, as in Yoshida's (1913) case.

The number and arrangement of the genital pores are subject to considerable variation. In most cases there is a single pore in each segment, located on the margin of the unpaired wing. Thus all pores are unilateral, an arrangement in striking contrast with the normal arrangement in the genus *Taenia* in which the pores are irregularly alternate. There are, however, many exceptions to this rule. In Bremser's (1819) case of *T. saginata*, according to Rudolphi (1819) the genital pore was in most cases located on the unpaired wing, but three variations were seen: (1) genital pore not on the unpaired wing but on the edge of one of the paired wings; (2) genital pore on the unpaired wing and on the edge of one of the two paired wings; (3) genital pore on the unpaired wing and on each of the paired wings. Two proglottids each had two genital papillae on the same unpaired wing, one located anteriorly, the other posteriorly. In Küchel's (1892) case, according to Vigener (1903), who re-examined his material, one segment bore three papillae and several segments had two sexual openings. As a rule there was but one sexual opening to each proglottid on the edge of any one of the three wings. In Bork's (1891) case although the unpaired ridge was papilliferous throughout, a supernumerary proglottid had a genital pore in the crevice between two of the wings. In Yoshida's (1913) case of *Taenia taeniaeformis* (*T. crassicollis*), "the genital pore is usually single in each segment, situated on any one wing of the worm, but there are sometimes two genital pores lying respectively on any two wings of the segment". In von Linstow's (1892) case of *Bothriocephalus tectus*, the genital pores are all situated on the middle line of the ventral surface, the normal position for cestodes of this genus.

The triradiate form in cestodes is not infrequently associated with the more common anomalies of supernumerary proglottids, forking, and fenestration. Both forking and supernumerary proglottids were observed by Vigener (1903) and Cattaert (1899). In the cases of Cattaert (1899) and Coats (1891), the worm ends in a triple fork, each branch forming one wing of the triradiate strobila. In McCulloch's (1913) case both fenestrated and supernumerary segments were frequent. The fenestration involved only mature segments, usually extending through two adjacent segments and following the line of the unpaired ridge. Asymmetrical segments were formed by the line of division between segments extending through only one or two of the three lateral wings, the opposite wing or wings being equal in length to the sum of these asymmetrical segments.

INTERNAL ANATOMY

The internal anatomy of polyradiate cestodes does not as a rule, present any special variation from the normal except in so far as the arrangement of the organs is associated with the peculiar external form. The extra wing or wings are as fully developed internally as the normal part of the cestode. The uterus, having its main stem running through the center of the polyradiate proglottid, sends off lateral branches into each of the wings irrespective of their relative size. Thus in Jelden's (1900) case, although the unpaired wing is here reduced to a mere ridge, it contains its full share of the uterine branches. The longitudinal excretory canals and longitudinal nerves, which in normal taeniae extend along each lateral margin of the cestode, are, in polyradiate forms, found in the same relative position in each of the wings. Several minor variations from the normal have been recorded. Both Neveu-Lemaire (1900) and Cattaert (1899) observed that the transverse muscle fibers at the point where the three wings separate occasionally formed a partition wall separating one wing from the other two. In Neveu-Lemaire's (1900) case the longitudinal canal in the unpaired ridge was larger than the other two. The ovaries lay in the posterior portion of the proglottid in the center of the "Y," ramifying into the two equal wings but not into the unpaired wing. Yoshida (1913) finds that in his specimen of *Taenia taeniaeformis*, the testes are distributed throughout the three wings and not confined to the dorsal side as in normal specimens of this species. The eggs of triradiate cestodes are usually reported as normal, but Küchel (1892) reports that out of ten eggs which he examined, one was normal, seven had eight hooklets arranged in pairs, and two had ten hooklets including one that was very small and incompletely developed.

THE WRITER'S CASE OF A TRIRADIATE TAENIA PISIFORMIS

Although Railliet (1892) has reported a case of *Cysticercus pisiformis* having six suckers, no case of an adult triradiate cestode of this species has yet been published. The present example was found in a mass of tapeworms expelled by an imported collie dog held at quarantine in Athenia, N. J., and treated with a taeniafuge for tapeworm infestation, which had been diagnosed from a microscopic examination. The mass of tapeworms received at this laboratory consisted of a great many fragments which were roughly estimated as belonging to from seventy-five to one hundred individuals, all of which, as far as examined, were of the same species, *Taenia pisiformis*. Although the entire mass was examined in a petri dish, no scolices were found. The identification of the species was verified by feeding experiments on a

rabbit. In this mass a number of chains of triradiate proglottids were found, the longest piece being 23 cm. representing the anterior half of the worm, except the head. In all about 52 cm. of the worm were recovered.

The parasite is uniformly triradiate throughout its entire length, the three wings being of almost equal size and having the same angle between them (Fig. 1). The wings are thickened at the base, thus maintaining the symmetry of the figure. Owing to shrinkage from

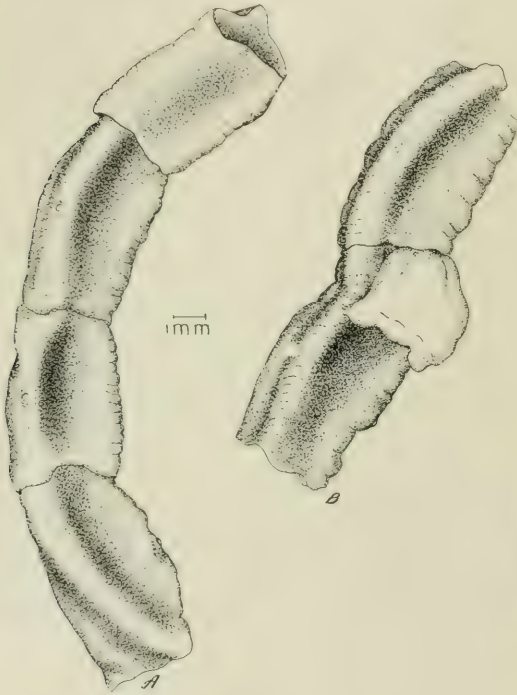


Figure 1

Fig. 1.—*A*. Portion of a triradiate *Taenia pisiformis*, showing the twisting of the strobila, and consequent displacement of the papilliferous wing. *B*. Another portion of the same specimen showing a supernumary proglottid without genital pore. [Original.]

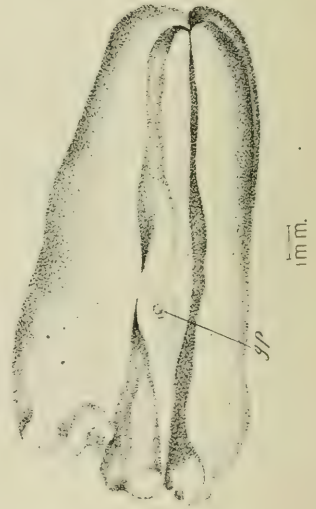


Figure 3

Fig. 3.—A tetraradiate proglottid of *Taenia saginata*. *gp*, genital pore. [Original.]

the formalin in which the specimen was sent, the genital pores are very difficult to observe in unmounted proglottids. As far as could be determined, however, there is but one pore to each segment, and it is always on the edge of the same wing. Owing to a spiral twist extending irregularly throughout the greater part of the strobila, the papilliferous wing of a given segment is seldom in line with the same wing in the

adjacent segments. Thus in Figure 1 A, while the two middle segments have the papilliferous wing on top, in the bottom segment it is on the right-hand side, while in the upper segment it is underneath. That this shifting of the papilliferous wing is due to the spiral twist and not to the fact that the pore may be on any one of the three wings, is made evident by finding the pores all in a straight line in those parts of the strobila not affected by the spiral twist. The longest proglottid was 13 mm. long by 2.5 mm. wide. The average was 7 mm. by 3.5 mm. Only one supernumerary proglottid was seen (Fig. 1 B). This was

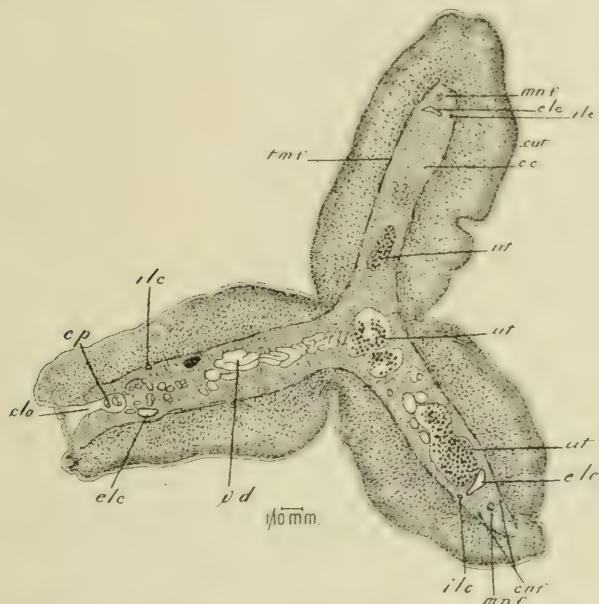


Fig. 2.—Cross section through a gravid proglottid of a triradiate *Taenia pisiformis*, in the region of the genital pore. *cc*, calcareous corpuscles; *clo*, cloaca; *cnf*, concomitant nerve fasciculi; *cp*, cirrus pouch; *cut*, cuticle; *elc*, external longitudinal canal; *ilc*, internal longitudinal canal; *mnf*, medullary nerve fasciculus; *tmf*, transverse muscle fibers; *ut*, uterus; *vd*, vas deferens. [Original.]

interpolated between two normal segments and affected only one wing, as the line of demarcation between it and the usual triradiate proglottid did not extend through all three wings. The supernumerary proglottid had no genital pore.

As in the other cases of triradiate cestodes, the sexual organs are found in all three wings. In ripe proglottids the uterus is seen to occupy the central portion of the body (Fig. 2), sending out branches into each wing. The eggs appear in all respects normal. Two longitudinal canals, the larger external and the smaller internal, appear in

each wing near the margin. The relative position of these canals varies both in different proglottids and in the different wings of the same proglottid. In the drawing (Fig. 2), the canals occur laterally, the ventral canal being much the larger. The principal longitudinal nerve, the medullary fasciculus, is between these canals and the margin of the wing, one in each wing. In one wing two accessory fasciculi could be seen on either side of the medullary fasciculus. The vas deferens at the plane of the section drawn occupies most of the medullary layer of one of the wings (Fig. 2), and, extending into the cloaca, passes between the two longitudinal canals. In mature proglottids the testes are distributed scatteringly throughout the three wings, being less numerous in the region of the vas deferens. As in normal proglottids, the ovaries occupy the central portion of the posterior half, and send out ramifications in all directions. The arrangement of the transverse and longitudinal muscle fibers, calcareous corpuscles and all other organs are, as far as observed, no different from that seen in normal cestodes of this species.

TETRARADIATE AND PENTARADIATE CESTODES

Only one case (Rosenberger, 1903) of an adult cestode having more than three wings throughout its strobila, has thus far been reported. That such an anomaly might exist was, however, anticipated by Railliet (1899), who examined a number of scolices of *Coenurus serialis* and found specimens having suckers ranging in number from three to ten. Railliet states in conclusion: "If the rule appears well established that a *Taenia* larva with six suckers will produce a worm with a triradiate chain, what malformation will arise from scolices having 3, 5, 8, 9, and 10 suckers?" In view of the assumed relation between the number of suckers of the scolex and the wings of the strobila, it is reasonable to suppose that Rosenberger's (1903) case of a pentaradiate *Taenia saginata* developed from a cysticercus with ten suckers, and that the present writer's case of a tettraradiate *Taenia saginata* was derived from an eight-suckered cysticercus.

Rosenberger (1903) received from a physician in Colorado a section of several proglottids of *Taenia saginata* having a "star-shaped" figure. The specimen was sent to Dr. Mohler of this bureau. Rosenberger's (1903) brief note includes a figure showing a chain of four proglottids having four equal or subequal wings radiating from a common center. As Rosenberger does not give the number of wings to his specimen and merely characterizes it as "star-shaped," the writer asked Dr. Mohler for further information. Dr. Mohler stated that according to his recollection there were five wings radiating from a common

center, giving the star-shaped figure described, and that the fifth wing did not appear in the drawing since it was hidden from view by the other wings. No detailed study was made of the specimen and the number and arrangement of the genital pores was not observed. Dr. Mohler was under the impression that the specimen had been deposited in the Helminthological Collections of the United States National Museum, but it could not be found. In looking through the material, however, a tetraradiate specimen was found, described below.

This specimen (No. 3269, Helminthological Collections, United States National Museum) consists of one proglottid only. The material was determined by Stiles in 1901 and collected the same year. Except for the name of the species (*Taenia saginata*) and the host,

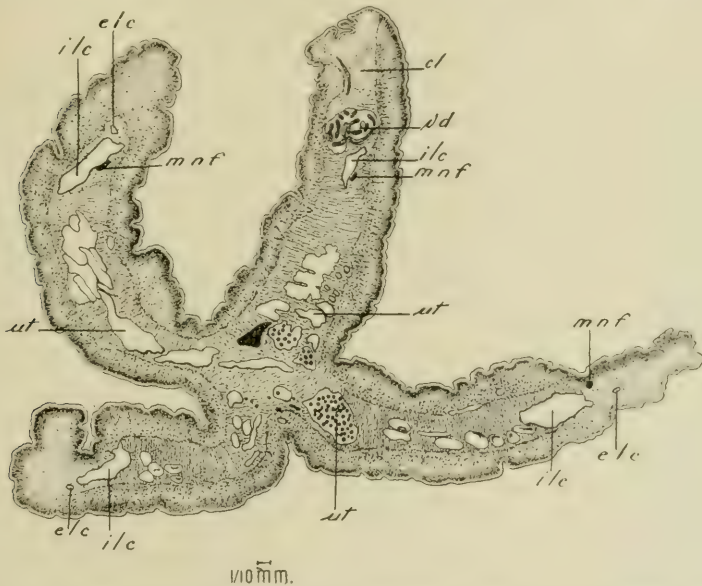


Fig. 4.—Cross section through a tetraradiate proglottid of *Taenia saginata*, in the region of the genital pore. *cl*, cloaca; *elc*, external longitudinal canal; *ilc*, internal longitudinal canal; *mnf*, medullary nerve fasciculus; *ut*, uterus; *vd*, vas deferens. [Original.]

no further information is given on the label. The proglottid is 15 mm. long with a maximum width of 8 mm. at the posterior extremity, which is considerably wider than the anterior end (Fig. 3). Three of the wings are of fairly equal width, the fourth wing, largely concealed in the drawing (Fig. 3), is considerably shorter than the others. There is but one genital pore, placed somewhat posterior to the middle of the segment, on the edge of the middle one of the three equal wings.

In cross-section (Fig. 4) the wings are seen to form an asymmetrical tetraradiate figure, the gravid uterus extending into each wing.

A large internal excretory canal of irregularly triangular outline is seen near the external edge of each wing. The smaller external canal appears between the internal canal and the outside edge. The uterine branches extending into all four wings are in most sections devoid of eggs and appear as large irregular lacunae in the medullary layer. A few eggs, however, appear in the main uterine stem (Fig. 4). The principal longitudinal medullary fasciculus is seen close to the wall of the internal canal, apparently flattened out by pressure from the adjacent longitudinal canal. The coiled vas deferens and the outline of the cloaca is seen in the middle of the three subequal wings.

ORIGIN OF POLYRADIATE CESTODES

Triradiate cestodes are sometimes referred to as being a fusion of two normal individuals; it would seem more logical, however, to consider them as representing the fusion of one cestode with half of another individual, since we invariably find six suckers to these triradiate forms and not eight, which we would expect if two individuals were blended. If, however, there were a true fusion we should expect to find a line of union, which does not appear in cross-sections. Moreover, if a cestode having irregularly alternate papillae were joined to another individual, we should expect to find about half of the proglottids with two genital pores and half with only one, yet it is usual to find only one genital pore to a segment, and that on the same wing throughout the strobila. From the fact that cysticerci with six suckers are occasionally found, and that oncospheres with more than six hooklets have been observed, it was suggested by Davaine (according to Railliet, 1899) that the cause of this abnormality originated in the egg. This view is, however, disputed by Leuckart (1880) and Railliet (1892); who point to the fact that in a coenurus, several of the scolices may have an abnormal number of suckers while the others are normal, yet all must have originated from the same oncosphere.

FEEDING EXPERIMENTS WITH TRIRADIATE TAENIA PISIFORMIS

In view of the fact that oncospheres with eight hooklets and cysticerci with six suckers have been found, it seems reasonable to expect that these forms would originate from a triradiate tapeworm, and Küchenmeister and Zürn (1878-81) and Railliet (1892) have suggested the advisability of feeding experiments to determine their origin. These authors were, however, unable to carry out the suggestion from lack of material.

Although the triradiate *Taenia pisiformis* described by the present writer was shipped in a solution of formalin of unknown strength, and kept in a 2 per cent. solution of formalin for one week after it

TABULAR LIST OF CASES OF POLYRADIATE CESTODES*

Author	Date	Species	Locality	No. of Specimens	Appearance of Head	Shape of Strobila
Andry	1741	<i>T. saginata</i> ?	France	1	Unknown	Triradiate?
Rudolphi	1810	<i>Dipylidium caninum</i>	Germany?	1	Normal	Triradiate
Brera	1810	<i>T. saginata</i> ?	Switzerland?	1	Unknown	Triradiate?
Bremser	1819	<i>T. saginata</i> ?	Austria?	1	Missing	Triradiate
Bremser	1819	<i>T. taeniaeformis</i>	Austria?	1	6 suckers	Triradiate
Levacher	1841	<i>T. saginata</i> ?	France	1	Unknown	Triradiate
Siebold	1853	<i>T. echinococcus</i>	Germany?	1	6 suckers	Triradiate
Küchenmeister	1855	<i>T. coenurus</i>	Germany?	2	6 suckers	Triradiate
Küchenmeister	1855	<i>T. saginata</i>	Cape Good Hope	1	Missing	Triradiate
Zenker	1861	<i>T. solium</i>	Germany?	1	6 suckers	Triradiate
Krause	1863	<i>C. cellulosae</i>	Germany	1	6 suckers
Cobbold	1866	<i>T. saginata</i>	England	1	Missing	Triradiate
Vaillant	1870	<i>T. saginata</i> ?	France	1	Missing	Triradiate
Cullingworth	1873	<i>T. saginata</i>	England	1	Missing	Triradiate
Küchenmeister & Zürn	1878-1881	<i>C. cerebrealis</i>	Germany	2	6 suckers
Leukart	1880	<i>T. coenurus</i>	Germany	1	6 suckers	Triradiate
Leukart	1880	<i>T. saginata</i>	Germany	1	Missing	Triradiate
Laker	1885	<i>T. solium</i>	Germany	2	6 suckers	Triradiate
Trabut	1889	<i>T. saginata</i>	Tonkin	1	6 suckers	Triradiate
Neumann	1890	<i>Anoplocephala perfoliata</i>	France	1	6 suckers	Triradiate
Coats	1891	<i>T. saginata</i>	Scotland	1	Missing	Triradiate
Bork	1891	<i>T. saginata</i>	Germany	1	Missing	Triradiate
Railliet	1892	<i>C. pisiformis</i>	France	1	6 suckers
Küchel	1892	<i>T. saginata</i>	East Africa?	1	6 suckers	Triradiate
v. Linstow	1892	<i>Bothriocephalus tectus</i>	{ S. Georgia	Several	Missing	Triradiate
			{ Antarctic reg.			
Monticelli ...	1893	<i>T. saginata</i> ?	Italy?	1	Missing	Triradiate
Barrois	1893	<i>T. saginata</i>	France	1	Missing	Triradiate
Pittard (after Railliet) ...	1895	<i>Bothriocephalus latus</i>	England?	1	Unknown	Triradiate
Shennan	1898	<i>T. saginata</i>	Scotland	1	Missing
Klepp	1898	<i>C. cellulosae</i>	Germany	1	6 suckers
Zürn	1898	<i>C. tenuicollis</i>	Germany	Several	6 suckers
Railliet	1899	<i>C. serialis</i>	France	Several	2, 3, 6, 8, 10 suckers
Cattaert	1899	<i>T. saginata</i>	France	1	Missing	Triradiate
Neveu-Lemaire	1900	<i>T. saginata</i>	France	2	1 case none
Jelden	1900	<i>T. saginata</i>	Germany	1	6 suckers	Triradiate
Lohoff	1902	<i>C. bovis</i>	Germany	1	6 suckers
Vigener	1903	<i>T. saginata</i>	Germany	1	6 suckers	Triradiate
Rosenberger ..	1903	<i>T. saginata</i>	Colorado, U.S.A.	1	Missing	Pentaradiate
Galli-Valerio ..	1909	<i>Coenurus serialis</i>	Switzerland?	1	6 suckers
MacCallum ..	1912	<i>T. saginata</i>	Canada	1	Missing	Triradiate
Yoshida	1913	<i>T. taeniaeformis</i>	Japan	1	6 suckers	Triradiate
McCulloch	1913	<i>T. saginata</i>	Missouri, U.S.A.	1	Missing	Triradiate
Foster	1915	<i>T. saginata</i>	U. S. A.?	1	Missing	Tetraradiate
Foster	1915	<i>T. pisiformis</i>	Europe	1	Missing	Triradiate

* In those cases where the species has been imperfectly described so that there is some doubt whether the cestode seen belonged to *Taenia saginata* or *T. solium*, the cestode is assigned to the species *saginata* followed by an interrogation point, as this species is the more numerous in most countries. Where the writer has failed to state the country from which the worm was collected, the locality given is that of the country in which he lived when the case was published (as far as could be determined). These doubtful localities are also marked by an interrogation point. In the last column marked "Shape of Strobila," the interrogation points indicate that the specimens were so imperfectly described that it is not certain whether they were triradiate forms or not. The dotted lines in this column indicate that the cestodes described are larval forms and hence have no strobilae.

was received, it was determined to use some of the material for feeding experiments. The writer was encouraged in the hope that the vitality of the eggs would prove unaffected by the formalin, from the fact that on several previous occasions he had fed to rabbits, proglottids which had been shipped in formalin and had always succeeded in infesting the animals. In the previous cases, however, the feeding experiments were performed as soon as the material was received.

A rabbit reared at the experiment station of the Bureau of Animal Industry was fed May, 1914, with two proglottids of the triradiate *Taenia pisiformis* already described. The rabbit died June 4, 1915. The postmortem revealed seven cysticerci, three of which were attached to the omentum, the others lying loose in the body cavity. The cysticerci were all fully grown and surrounded by a protective membrane, the largest cyst measuring 2 cm. long by 1 cm. in diameter. In dissecting out the invaginated scolices to determine the number of suckers, two specimens were mutilated and the number of suckers could not be positively determined. There is no reason to suppose, however, that more than the usual number of suckers were present. The other five specimens were entirely normal.

It can not be positively demonstrated that the rabbit was uninfested with *Cysticercus pisiformis* at the time it was fed. On the other hand, the fact that the rabbit was reared and kept in a cage until its death, and that as far as the writer is aware no rabbits from this source have been found infested with *C. pisiformis* unless as the result of feeding experiments, is very strong evidence for assuming that the cysticerci found resulted from the feeding experiment and not from a previous infestation.

The experiment therefore failed to prove that cestode larvae with an excessive number of suckers are the offspring of polyradiate adults. On the other hand, it appears that a triradiate cestode may give rise to perfectly normal larvae, which presumably would develop into normal adults. Whether or not cestode larvae with an excessive number of suckers have any genetic connection with polyradiate adult cestodes, is a question still remaining unanswered.

SUMMARY

1. The term polyradiate is used to designate those cestodes whose strobila is uniformly divided into three or more rays or wings extending throughout the entire strobila and radiating from a common axis, and whose scolices have two suckers for each of the rays present. Presumably the larvae of these polyradiate forms have as many suckers as appear in the scolices of the adults.

2. Altogether forty-four cases of polyradiate cestodes (including larvae) have been reported, in all but two of which the adult forms were triradiate. The greater number of cases are triradiate forms of *Taenia saginata*, but several species are represented and they are found in widely distributed localities. The greater frequency of this anomaly in *T. saginata* is probably due to the greater chances for observation of this species.

3. Of the two cases having more than three rays, one is apparently pentaradiate (Rosenberger's case), and the other is tetraradiate (Foster's case, the present paper); both are specimens of *Taenia saginata*. Since triradiate forms are assumed to originate from larvae with six suckers, it is suggested that the tetraradiate and pentaradiate forms originated from cysticerci having 8 and 10 suckers, respectively, larvae with this number of suckers having been found by Railliet (1899) in the case of *Coenurus serialis*.

4. The view that the origin of polyradiate forms can be traced to the ovum, is supported by the finding of oncospheres having an excessive number of hooklets. On the other hand, this view is disputed by the finding of both normal and abnormal scolices in the same coenurus. A feeding experiment with triradiate proglottids of *Taenia pisiformis* tends to show that in this species perfectly normal cysticerci may result from abnormal adults. Whether or not cysticerci with an excessive number of suckers and oncospheres with an excessive number of hooklets have any genetic connection with polyradiate adults, is a question which has not yet been solved.

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SOME NOTES AND EXPERIMENTS ON *SARCOCYSTIS*
TENELLA, RAILLIET *

JOHN W. SCOTT

In a recent paper on Sarcosporidia encountered in the Canal Zone, Darling (1915) makes the interesting suggestion that the "Sarcosporidia may be side-tracked varieties of some of the Neosporidia of invertebrates which have invaded the musculature of a hospitable though by no means definitive host and are unable to continue further their life cycle and escape from a compromising and aberrant position." Darling thinks the high incidence of infection in cattle, sheep, swine, and horses favors this view, and points out the ease with which such animals may obtain Neosporidia in their food. In this connection the writer has obtained during the past year some data concerning the sheep sarcocyst that appear to favor a similar conclusion. Though the results of the experiments were negative the inferences are nevertheless interesting.

A large percentage of the range sheep of Wyoming are probably infected with *Sarcocystis tenella*. Out of sixty-five sheep examined at different times at a local slaughter house fifty were found infected, or nearly 77 per cent. In some lots approximately 100 per cent. were infected. The parasite presented the general appearance described by Railliet (1895), Alexieff (1911), and others. The stages found usually showed an alveolar structure, a few presented the pansporoblast stage, but none were in the Balbian stage. Some of the largest cysts, and presumably the oldest ones, had the appearance of fading out or undergoing degeneration, and so far as one could observe from living material there was no indication that the parasites ever escape from the muscle of the host. The heart muscle and the esophagus were examined for the cysts, but they were found much more frequently in the heart.

Since the direct observations just mentioned gave no clue to the life history, a preliminary series of experiments was planned to test various hypothetical methods of infection. Smith (1901) has succeeded in getting a direct infection in mice by feeding muscle tissue from infected mice, and the first experiment was to determine if this method would succeed in the case of the sheep sarcocyst. While Smith's experiment might account for a natural transmission of the

* Contribution No. 2 from the Laboratory of Zoology and Parasitology, University of Wyoming. Experiments by aid of Adams Fund.

Sarcosporidia of carnivorous animals, it cannot explain transmission in the sheep, or in other herbivora. Dr. L. D. Swingle, formerly of the University of Wyoming, fed five young lambs pieces of heart muscle containing the sarcocysts. From the time they were born these lambs were fed dry feed in a dry lot and were watered from the city water supply which has its source in deep springs. When the writer examined these lambs at slaughtering time not a single one was infected. Control lambs, kept under the same conditions, were likewise uninfected. This experiment has been repeated with similar results, and it appears to show that the parasites in the sarcocysts are not in a transmissible stage or condition, using the direct method.

Several authors have held the view that an intermediate host is necessary, since the delicate structure of the spores of Sarcosporidia would ill adapt them to withstand the conditions outside of the vertebrate host. For this reason Wasielewski (1896) believes that, as in the case of the malarial parasite, an intermediate host is necessary in order to convey the parasite from one host to another. On this theory, Minchin (1903) suggests three possibilities in regard to infection by Sarcosporidia: (1) The intermediate host is a large carnivore, as the dog, in case of parasites like those of the pig or sheep; (2) after death the parasites are taken up by some carrion-feeding animal, which might be some vertebrate, as bird or mammal, or some invertebrate, as blow-fly or carrion-beetle; (3) the infection might be taken on by some internal parasite, for example, a flatworm or a nematode. Minchin regards the third explanation as unlikely, and I have frequently found lambs with no parasitic worms present, but at the same time infected with Sarcosporidia. He believes the second explanation receives some support from the extremely toxic nature of the parasites themselves, since this would aid in the death of the host.

More recently Minchin and Thompson (1915) have shown that the rat-trypanosome passes through a cycle in the digestive tract of the rat-flea, and that after this period the rat becomes infected either by licking the flea feces or by ingesting the flea. While there is no blood-sucking parasite of the sheep in Wyoming that could perform a similar rôle for *S. tenella* and at the same time account for the prevalence of this parasite, the object of the following experiment was to test whether digestion by a carnivorous animal (coyote or dog) would render the Sarcosporidia contaminative.

My second experiment, therefore, was essentially a test of Minchin's first hypothesis. On August 28 a young dog was fed liberally on sheep hearts containing sarcocysts and placed in a wire cage where the grass had not been pastured. On August 31 the dog was again fed with infected muscle, and in the afternoon of September 2 he was taken out

of the cage. By this time feces were well scattered over the grass. Water was sprinkled heavily on the grass September 3 and 9 in order to further facilitate contamination. On September 11 two lambs, previously kept in a dry lot, were placed in the cage and left for about thirty-three hours. Again October 1 two more lambs were put in the cage and left for twenty-four hours. A later examination showed that none of these lambs was infected.

Though the experiment is not conclusive, the evidence is against the idea that the digestion of muscle tissue by a carnivorous animal is the normal method by which the Sarcosporidia of the sheep are set free in order that they may be accessible through its own herbivorous habits. Indeed, the comparatively rare occasions on which coyotes and dogs obtain sheep's flesh would hardly account for the frequency with which flocks are infected.

During the summer of 1914 another experiment was tried that is even of more interest, and to a certain extent is a test of the second explanation suggested by Minchin. Two groups of lambs were used. Group one, consisting of twenty-one ewes and eighteen lambs, was allowed to graze in pasture A, where both dry and swampy conditions were present. In this pasture is located a permanent pond fed by seepage water, and the pond overflows except in the dry season into the swamp. The swampy portion of the pasture went dry about mid-summer. Group two, consisting of twenty-five ewes and twenty-three lambs, was allowed to graze in a small dry pasture B, where no water was present except such as fell in the frequent though scanty summer rains. At several times during the summer infected pieces of heart muscle were scattered in the pond in pasture A, and also upon the grass in pasture B. When the lambs were killed 55 per cent. of Group 1 (ten out of eighteen) were found infected, and 21 per cent. (five out of twenty-three) of Group 2. From this result it appeared (1) that lambs may become infected either through water or by eating infected grass; or, (2) which is more probable, the infection was independent of the experiment.

On the assumption that the third experiment gave positive results, it is hard to reconcile the infection of lambs in Group 2 with the results from the first two experiments. For if one does not get positive results by direct feeding of infected muscle, or by feeding grass contaminated with feces after the ingestion of infected muscle, one would not expect any infection where the muscle was simply scattered on the grass. Again, if scattering muscle containing sarcocysts on the grass or in water, and the consequent decay is a necessary condition for infective material, we are confronted with the fact that the natural death and decay of sheep carcasses will not account for all cases of infection. It

therefore appears that the infection which took place under the conditions of the third experiment was independent of the experiment. What other possibilities are left?

If we assume that the sheep is the definite host of *S. tenella* and that no intermediate host is necessary, we must conceive that the parasites are set free in the blood, find their way to the exterior in the excretions, probably in feces or urine, and that in this way food or drink is contaminated. Most authors regard this as extremely improbable and we have already stated that there is no evidence to show that the Sarcosporidia are freed in this way. Again, lambs fed along with ewes in a small dry lot must frequently have the hay contaminated with feces or urine, and yet under these conditions in the experiments no infection has so far occurred. On the other hand, if we consider an intermediate host is necessary, three possibilities arise. We may think of an external blood parasite as being the active agent in transferring the Sarcosporidia from one sheep to another; but in this case no external parasite appears to satisfy all the conditions, and we ought to get transmissions in a dry lot as well as elsewhere. Second, we may think of the intermediate host as feeding upon contaminated feces or urine and later depositing the spores on food or drink of the sheep. We can at once eliminate the matter of drink, for there was no water to be had in pasture B of my third experiment where infection occurred. While certain insects on this hypothesis might possibly account for the infection of both groups of the third experiment, it is probable that control lambs, confined with their ewes in an adjacent dry lot, would also have become infected, and we have already noted that there is no evidence that spores escape in the way suggested. The third possibility would be to assume that the intermediate host is a carrion-feeding animal. But no carrion-feeding vertebrate common to pastures A and B could be discovered. Besides, the heart muscle thrown into the shallow pond in pasture A sank to the bottom, where it was inaccessible to any sort of carrion-feeding animal, especially any insect, that might happen to be present. Minchin's second suggestion, therefore, as a possible method of infection appears to be out of the question.

If, however, the sheep is not the definitive host of *S. tenella*, but the presence of the parasite is more or less accidental, the results of the experiments given are more easily explained. First, it is evident that the conditions for infection are much more favorable in pasture A than in pasture B, though the parasites were acquired in both places. It is therefore well to consider further the difference between the two places and their similarities. In pasture B the grass was rather sparse, flowering plants were few in both numbers and kind, and various flies,

ants, beetles (under cattle dung), mosquitoes, bees (occasionally), grasshoppers, a few moths, and spiders were present. All of these things were present in pasture A, and, besides certain specific water animals, it differed from pasture B in the following particulars: There were more flowering plants, sedges were abundant in the swampy portions, mosquitoes and various flies were very abundant, and bees and moths were more frequently found. Now Erdmann (1910), Minchin (1912), and others look upon the *Sarcosporidia* as one group of *Neosporidia*, on the basis of what is already known in regard to their development and structure. If we accept their conclusions, and if according to Darling's suggestion the *Sarcosporidia* are aberrant *Neosporidia*, and infection of herbivora is acquired by accidental ingestion of invertebrate hosts or by the ingestion of the droppings of such hosts upon flowers or leaves, we can readily understand how infection took place in both pastures and how the percentage of infection in pasture A greatly exceeded that in pasture B. Further experiments are now in progress which will test the question of whether *S. tenella* is in reality only an aberrant form of one of the *Neosporidia*.

SUMMARY

To sum up this paper, one may say that the experiments are chiefly important for their negative significance. Infection with *S. tenella* failed to occur, (a) as the result of feeding infected muscle, (b) as the result of eating grass contaminated with feces from a carnivorous animal previously fed on infected muscle, and (c) by allowing infected muscle to decay either on dry grass or in a pond. The apparently positive results of the third experiment are best explained as due to conditions independent of that experiment. All of the evidence favors the view that the sheep is not the definitive host of *S. tenella*, and therefore is in accord with Darling's suggestion that the muscle parasites of vertebrates are aberrant forms.

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EGG VARIATION IN A TREMATODE SPECIES

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In 1903 H. B. Ward (1903a) called attention to the importance of the eggs in determining human entozoa. Later the same writer in a paper devoted entirely to the eggs of human parasites (Ward, 1908) emphasizes his earlier view and on page 180 comes to the following conclusion in regard to recorded observations of egg size: "The very existence of marked variation [in egg size] in the records of a single form is presumptive evidence that in the absence of errors of observation, two or more species are confused under the single appellation."

In all of Looss' extensive work on the trematodes he uses the size of eggs as a character of specific value, and Lühe (1909) in his summary of the fresh-water trematodes of Germany gives the egg size in his account of almost every species. These and other workers on trematodes have generally recognized the importance of determining the limits of variation and the average size of ripe, normal eggs among trematode species. On that account the following record of a considerable variation from the species average in the size of the eggs from three individuals of one of the common frog lung flukes seems worthy of note.

Recently while working on the anatomy of *Pneumonoeces similiplexus* Stafford from the lung of the leopard frog *Rana pipiens* more than two hundred eggs from ten different individuals were measured to determine accurately the average egg size for the species. The average length of the eggs for this species as computed from these measurements was found to be $37.6\ \mu$, and the range of variation showed a minimum of $34\ \mu$ and a maximum of $40\ \mu$. Later, while examining three good-sized specimens of the same species from a single frog from Oshkosh, Wisconsin, I was struck by the fact that the eggs appeared smaller than in the forms previously examined. Measurements of two hundred eggs from these individuals confirmed this opinion, since the average egg length was found to be only $34.2\ \mu$ and the limits of variation from 30 to $37.4\ \mu$. This gives a difference between the Oshkosh flukes and the normal egg average length of the species *Pneumonoeces similiplexus* of $3.4\ \mu$, or a greater difference than is found between two distinct species of this same genus, viz., *Pneumonoeces breviplexus*, with an average egg length of $22.5\ \mu$, and *Pneumonoeces longiplexus*, with an average of $24.8\ \mu$. A study of the three individuals with the

egg variation showed that in the rest of the characters used for specific diagnosis they agreed with my other specimens of *Pneumonoeces similiplexus*. This observation shows that a distinct variation may occur within a species in a character which has proved to be generally constant.

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SOME NEW GREGARINE PARASITES FROM ARTHROPODA *

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For several years I have been studying a number of gregarines parasitic in various arthropods. The literature apparently contains no record of most of them and consequently they are here described as new species. A few of the species studied were already known, but I am able to give new records on distribution and additional data on biology and life-history. Careful attention was devoted to the biology of the forms studied and extended experiments were conducted on life-history problems. In this paper is presented a brief account of these studies, which will be published in full at a later date. Especial attention is called here to the observations on movement in gregarines and on cyst formation. The descriptions of new species though concise have been worked over so carefully that it is thought they will be ample for accurate determinations.

BIOLOGICAL OBSERVATIONS

The polycystid gregarines have a septum which divides the cell into two or more distinct compartments, and the species described in this paper are all of this type. Polycystid gregarines inhabit chiefly the mid-intestines and intestinal diverticula of arthropods and are often found in large masses comprising many hundred parasites. In some genera the adult animals are solitary; in others they are attached one behind the other. Most of the latter are biassociative, but a few genera occur in chains of from three to ten or twelve individuals.

The sporonts, or adult animals, move about freely in the lumen of the intestines or lie inert between the lobes. The trophozoites, or young individuals, live either entirely within the epithelial cells, as in the family Stenophoridae, or attached to the free ends of the cells by means of variously shaped epimerites, globular in the genus *Gregarina*. When a trophozoite has absorbed sufficient nourishment from the host cell, either directly through its walls or by means of the epimerite, it breaks forth from the shriveled cell and becomes a sporont, living free in the intestine, and the useless epimerite, if present, is gradually lost. The animal now receives its food entirely by absorption of the digestive juices direct from the host intestine.

* Contributions from the Zoological Laboratory of the University of Illinois, under the direction of Henry B. Ward, No. 48.

Movement of the free individuals takes place by a gradual progression and by bending of any part of the deutomerite. There are two structures necessary for motion. Running crosswise in the outer part of the endocyte is a delicate network of fibrillae, the myonemes, sometimes seen with a rather low power in individuals nearly devoid of protoplasm. In the outer portion of the epicyte, the outside layer of the body, there are found very fine longitudinal striations visible only with an oil-immersion lens. In the furrows between these striations there are minute pores (Schewiakoff, 1894) through which a gelatinous material is exuded. The animal progresses by contracting a few myonemes on that side of the body which happens to be ventral and this causes a minute undulatory motion against the slide. At the same time the animal secretes mucus, which enables it to move forward against friction, much as a slug moves forward by a wave-like motion on its ventral side. The trail of mucus left on the slide is the now useless material which has gradually been pushed backward through the longitudinal furrows by the progressing animal. Bending movement is effected by a contraction and expansion of the myonemes in any part of the deutomerite.

As the beginning of cyst-formation, two individuals, either associative or solitary, commence to revolve in a large circle. If the animals are solitary, an individual is drawn into the vortex of one which has started to revolve alone. If of a biassociative type, the two commence to revolve together. The spiral gradually becomes smaller as they continue in motion and the animals come to lie in contact laterally. Motion still continues and becomes rotary. As the two sporonts are forming a compact sphere, a thick, transparent covering is being laid down on the outside of the cyst which consists of as many thin layers of gelatinous material exuded from the posterior end of the moving animals as there are rotations before the animals come to rest. The fully formed cyst, sometimes still rotating, now passes from the mid-intestine of the host to the rectum and is given out with the feces.

Finding suitable moisture, the cyst develops within from 24 to about 48 hours with the formation and growth in many genera of as many as fourteen enormously long spore ducts. Each sporont breaks up into gametes, the gametes from one sporont uniting when fully formed with those from the other to form zygotes; and when the resultant spores have become mature they are forced out violently through the spore ducts into the surrounding medium. They become scattered and if accidentally eaten by an insect of the same species as the host, the outer spore wall is dissolved in the intestine, releasing eight active sporozoites. The latter pass to the epithelial cells

and either become attached or completely embedded, when the life-cycle begins anew.

There is evidence to indicate that auto-infection may occur by the cysts ripening in the intestine, and this would account for the enormous number of parasites often found within a single host.

The arthropods from which the parasites were taken include the Diplopoda, Orthoptera and Coleoptera, and the species are grouped in this order in the text. The gregarines described are members of the following genera: *Amphoroides*, *Steinina*, *Stenophora*, *Gregarina*, and a new genus which is here designated *Leidyana*.

GREGARINES IN THE DIPLOPODA

Infection in the diplopods is fairly heavy and about three fourths of the individuals examined at Urbana were parasitized. Parasites were abundant in the early spring as well as in the fall. Some species were nearly always found to be infected, others never.

Stenophora diplocorpa n. sp. (Fig. 1): Sporonts solitary, elongate. Maximum length 360μ , width 15μ . Ratio, length protomerite: total length : : 1:16 to 1:25. Ratio, width protomerite: width deutomerite : : 1:2 to 1:3. Protomerite dome shaped, widest at posterior margin and as wide as long. Slight constriction at septum. Deutomerite slender, elongate, incompletely divided into two nearly equal parts by a crosswise constriction, widest just anterior to this constriction. Cylindrical behind the constriction and broadly rounded at posterior end. Protomerite nearly transparent, deutomerite pale tan, not opaque. Nucleus visible in vivo, situated just behind constriction in the deutomerite, spherical, and containing one karyosome. Cyst and spores unknown.

Taken at Urbana, Illinois. Host: *Euryurus erythropygus* (Brandt). Habitat: intestine.

Stenophora impressa n. sp. (Fig. 2): Sporonts solitary, ellipsoidal. Maximum length 375μ , width 48μ . Ratio, length protomerite: total length : : 1:12. Ratio, width protomerite: width deutomerite : : 1:2.3. Protomerite conical, dilated in posterior half, as wide as high. An apparent pore at anterior end. Constriction at septum not deep. Deutomerite ellipsoidal, widest through central part, posterior extremity blunt or rounded. Endocyte of protomerite nearly transparent, of deutomerite opaque. Nucleus spherical with one large karyosome. Cysts spherical, 160μ in average diameter. Spores not known.

Taken at Urbana, Illinois. Host *Parajulus impressus* (Say). Habitat: intestine.

Stenophora lactaria n. sp. (Fig. 3): Sporonts solitary, elongate, ellipsoidal. Maximum length 480μ , maximum width 39μ . Ratio,

length protomerite: total length : : 1:10 to 1:16. Ratio, width protomerite: width deutomerite : : 1:1.2. Protomerite conical, dilated above base and tapering to a point. An apparent pore at apex. As broad as high. Constriction at septum. Deutomerite ellipsoidal, widest in anterior third, tapering to an acute, rounded extremity. Endocyte of protomerite nearly transparent, of deutomerite opaque. Nucleus ellipsoidal, twice as long as wide. Cysts spherical, 150 to 170 μ in diameter. Spores not known.

Taken at Urbana, Illinois. Host: *Callipus lactarius* (Say). Habitat: intestine.

Amphoroides calverti (Crawley) (Fig. 4): Sporonts solitary, elongate. Maximum length 1670 μ , average length 1400 μ , average width 120 μ . Ratio, length protomerite: total length : : 1:47. Ratio, width protomerite: width deutomerite : : 1:2.5 to 1:3. Protomerite greatly compressed in sporonts, shallow, five times as wide as high. Deep crater within top. Constriction at septum sharp and deep. Deutomerite elongate, widest in anterior third, tapering to a sharp point. Endocyte of protomerite tan in color, not dense; of deutomerite opaque, white. Nucleus small, spherical, not visible in vivo. Myocyte well developed. Cysts spherical, averaging 380 μ in diameter. Dehiscence by simple rupture. Spores not known.

Taken at Urbana, Illinois. Host: *Callipus lactarius* (Say). Habitat: intestine.

This species was described by Crawley (1903a) as *Gregarina calverti*, but the elongate shape of the sporonts, great size, dehiscence of the cysts by simple rupture, and the fact that all the animals are solitary prove that the species is not a member of the genus *Gregarina*. I place it in the genus *Amphoroides* because of the crateriform protomerite.

GREGARINES IN THE COLEOPTERA

The following nine species have been found in beetles and beetle larvae in the two general localities mentioned. In no instance has a complete life-history been established, but the generic position is determined beyond doubt by known characters. The members of the genus *Gregarina* are superficially very similar, but a close inspection yields points of difference sufficient to indicate the individuality of each species. While the primitives of several species are similar, the satellites are dissimilar and afford one means of differentiation. The relative sizes of the species and the color and density of the protoplasm afford other means of identification. The visibility of the nucleus is important in identification. The literature has been carefully investigated in the anticipation that some of the species, especially those in

the Elateridae and the Tenebrionidae had been previously described. All are, however, new.

Gregarina katherina n. sp. (Fig. 5): Sporonts biassociative, ellipsoidal. Length of associations 96 to 150 μ . Sporonts 45 to 70 μ long, 20 to 34 μ wide. Ratio, length protomerite: total length primate :: 1:6. Ratio, width protomerite: width deutomerite :: 1:7. Protomerite of primate dome shaped, of satellite flattened. Deutomerite ellipsoidal. Nucleus spherical, one large karyosome. Epimerite large, sessile, a hyaline knob. Cyst and spores not known.

Taken at Oyster Bay, Long Island, N. Y. Host: *Coccinella novum-notata* Herbst. Habitat: intestine.

Gregarina barbarara n. sp. (Fig. 6): Sporonts biassociative, ovoidal to subspherical. Length of association (average) 250 μ . Sporonts (primates) average 145 μ long, 90 μ wide. Ratio, length protomerite: total length primate :: 1:6. Ratio, width protomerite: width deutomerite :: 1:2.2. Protomerite hemispherical in primate, flattened in satellite, six times as wide as high, deutomerite ovoidal in primate, widest part in central region. Deutomerite of satellite widest in anterior third, no constriction at septum, contour here perfectly smooth. Nucleus small, spherical, with one karyosome. Body practically transparent. Cyst and spores not known.

Taken at Oyster Bay, Long Island, N. Y. Host: *Coccinella* sp. Habitat: intestine.

Gregarina globosa n. sp. (Fig. 7): Sporonts biassociative, subglobose. Length of associations 435 μ . Length of sporonts 260 μ , width 180 μ . Ratio, length protomerite: total length :: 1:8.6. Ratio, width protomerite: width deutomerite :: 1:2.4. Protomerite hemispherical, broadest at base, no constriction at septum. Deutomerite nearly spherical. Protoplasm dense, dark gray to black in primate, lighter in satellite. Nucleus spherical. Cyst and spores not known.

Taken at Urbana, Illinois. Host: *Coptotomus interrogatus* Fab. Habitat: intestine.

Gregarina monarchia n. sp. (Fig. 8): Sporonts biassociative, elongate cylindrical. Length of associations 570 μ , width 130 μ . Ratio, length protomerite: total length :: 1:7. Ratio, width protomerite: width deutomerite :: 1:1.2. Protomerite subspherical, widest through middle portion, constriction at septum. Deutomerite elongate cylindrical, equal in width throughout, broadly rounded posteriorly. Deutomerite dense, black in transmitted light. Protomerite nearly transparent. Nucleus not visible in vivo. Cyst and spores not known.

Taken at Urbana, Illinois. Host: *Pterostichus stygicus* Say. Habitat: intestine.

Gregarina intestinalis n. sp. (Fig. 9): Sporonts biassociative, broadly ellipsoidal. Length of associations 320μ . Maximum length of sporonts 160μ , maximum width 80μ . Ratio, length protomerite: total length :: 1:5. Ratio, width protomerite: width deutomerite :: 1:2. Protomerite subspherical, widest through middle portion, deep constriction at septum. Deutomerite broadly ellipsoidal, protoplasm dense, dark gray. Nucleus not visible in vivo. Cyst and spores not known.

Taken at Urbana, Illinois. Host: *Pterostichus stygicus* Say. Habitat: intestine.

Gregarina gracilis n. sp. (Fig. 10): Sporonts biassociative, elongate ellipsoidal. Maximum length of associations 370μ ; maximum length of sporonts 190μ , maximum width 75μ . Ratio, length protomerite: total length :: 1:8. Ratio, width protomerite: width deutomerite :: 1:2. Protomerite hemispherical. Deutomerite elongate ellipsoidal. Color gray. Nucleus not visible in vivo, spherical, small, with one karyosome. Cysts average 90μ in diameter. Spores not known.

Taken at Urbana, Illinois. Host: larvae of *Elateridae*. Habitat: intestine.

Gregarina tenebrionella n. sp. (Fig. 11): Sporonts biassociative, subglobose, very small. Maximum length of association, 140μ , average length 125μ . Ratio, length protomerite: total length :: 1:4. Ratio, width protomerite: width deutomerite :: 1:1.7. Protomerite dome shaped, deutomerite nearly spherical in primitive, ellipsoidal in satellite. Nucleus small, spherical. Protoplasm gray. Cyst and spores not known.

Taken at Urbana, Illinois. Host: larvae of *Tenebrionidae*. Habitat: intestine.

Gregarina fragilis n. sp. (Fig. 12): Sporonts biassociative, ellipsoidal. Length of associations 200μ . Maximum length of sporonts 110μ , maximum width 60μ . Ratio, length protomerite: total length primitive :: 1:5. Ratio, width protomerite: width deutomerite :: 1:2. Protomerite dome shaped, cylindrical in posterior third. Protomerite of satellite same shape but slightly flattened anteriorly. Deutomerite ellipsoidal. Nucleus small, spherical, with one karyosome. Body practically transparent. Cyst and spores not known.

Taken at Urbana, Illinois. Host: *Coccinella* sp. Habitat: intestine.

Steinina rotunda n. sp. (Fig. 13): Sporonts solitary, globose. Maximum length 250μ , maximum width 130μ . Ratio, length protomerite without epimerite: total length :: 1:2.3. Ratio, width protomerite: width deutomerite :: 1:1.1. Protomerite conoidal, dilated

at beginning of posterior two thirds, constricted at septum. Protomerite densest in posterior half. Deutomerite spherical to obovate, posterior end either rounded or slightly pointed. Nucleus large with one large karyosome in young, with many chromatic bodies in adult. Endocyte light brown. Epimerite spherical, hyaline, persistent on large animals free in lumen of intestine. Cyst and spores not known.

Taken at St. Joseph, Illinois. Host: *Amara angustata* Say. Habitat: intestine.

GREGARINES IN THE ORTHOPTERA

Five of the following species are new, one representing a newly created genus. New distribution records and new measurements are given for three species which are already known in the literature.

Gregarina nigra n. sp. (Fig. 14).—Sporonts biassociative, cylindrical. Maximum length of associations, 1000μ . Maximum length of sporonts 530μ , maximum width 180μ . Ratio, length protomerite: total length primate :: 1:4. Ratio, width protomerite: width deutomerite :: 1:1.4. Protomerite a truncate cone angular at the free corners. Width equal to height. Widest at base, no constriction or a very slight constriction at septum. Protomerite of satellite scarcely flattened. Deutomerite cylindrical, broadly rounded posteriorly. Endocyte black. Nucleus not visible in vivo, spherical, containing many small karyosomes. Cysts and spores not known.

Taken at Urbana, Illinois. Hosts: *Melanoplus femur-rubrum* (deGeer); *M. differentialis* (Uhler); *Encoptolophus sordidis* (Burmeister). Habitat: intestine.

Gregarina stygia n. sp. (Fig. 15): Sporonts biassociative, obese. Maximum length of associations 360μ , length sporonts 180μ . Primate and satellite of approximately the same length. Maximum width of primate 100μ . Ratio, length protomerite: total length primate :: 1:6. Ratio, width protomerite: width deutomerite :: 1:1.6 to 1:2. Protomerite hemispherical in primate, flattened in satellite. Deutomerite of primate broadly ellipsoidal, nearly as wide as long; of satellite widest in anterior half, tapering slightly. Nucleus small, spherical. Endocyte dark tan but not dense, nucleus visible in vivo in both primate and satellite. Sarcocyte thicker in both protomerites than in the deutomerites. Trophozoite with a simple, small, knobbed epimerite. Cysts 150μ in diameter. Spores not seen.

Taken at Cold Spring Harbor, Long Island, N. Y. Host: *Ceuthophilus stygicus* (Scudder). Habitat: intestine.

Gregarina galliveri n. sp. (Fig. 16): Sporonts biassociative, maximum length of associations 590μ ; maximum length of sporonts 300μ , width 130μ . Ratio, length protomerite: total length primate :: 1:5.

Ratio, width protomerite: width deutomerite :: 1.1:1. Protomerite flattened, broad and low. Three times as wide as high. Deutomerite of primate vase shaped, constricted at top, widening in posterior half. Deutomerite of satellite subspherical to ovoidal. Endocyte very dense in both protomerite and deutomerite, dark brown in color. Nucleus small, spherical, not visible in vivo. Cysts spherical, 350μ in average diameter. Spore ducts numerous. Spores not seen.

Taken at Oyster Bay, Long Island, N. Y. Host: *Gryllus abbreviatus* Serv. Habitat: intestine.

Gregarina illinensis n. sp. (Fig. 17): Sporonts biassociative, elongate cylindrical. Maximum length of associations 1100μ ; length sporonts 550μ , width 180μ . Ratio, length protomerite: total length primate :: 1:5. Ratio, width protomerite: width deutomerite :: 1:1.1 to 1.5. Protomerite dome shaped, slightly constricted at septum. Deutomerite elongate cylindrical, broadly rounded behind. Protomerite of satellite cupped at top for insertion of posterior end of primate. Nucleus large, spherical, with many small chromidial bodies. Endocyte dense, black in both protomerite and deutomerite. Cysts and spores not recovered from the host.

Taken at Urbana, Illinois. Host: *Ischnoptera pennsylvanica* (deGeer). Habitat: intestine.

Gregarina achetae-abbreviatae Leidy (Fig. 18): Sporonts biassociative, obese. Maximum observed length 500μ ; average sporonts 450μ long, 225μ wide. Ratio, length protomerite: total length primate :: 1:3. Ratio, width protomerite: width deutomerite :: 1:1.1. Protomerite hemispherical to subglobose, width twice the height. Slight constriction at septum. Deutomerite stout bodied, nearly as wide as long. Widest at shoulder where it is very little wider than protomerite. Posterior end truncate. Epimerite undescribed. Endocyte dense in deutomerite, less so in protomerite. Nucleus not visible in vivo and not seen. Cysts spherical, 250μ in average diameter. Spore ducts two to five in number, of maximum length 1000μ . Spores barrel shaped, $4.5 \times 2.25\mu$.

Taken at Haverford, Pa., and Urbana, Illinois. Host: *Gryllus abbreviatus* Serv. Habitat: intestine.

Gregarina rigida (Hall) Ellis (Fig. 19): Sporonts biassociative, stout bodied. Maximum length of associations 1425μ , average length 550μ . Sporonts 250 to 750μ long, 130 to 210μ wide. Ratio, length protomerite: total length of primate :: 1:3 to 1:6. Ratio, length protomerite: total length satellite :: 1:5 to 1:16. Ratio, width protomerite: with deutomerite :: 1:1.4. Protomerite somewhat flattened, width sometimes three times the height, generally less. Constriction at septum more or less indistinct. Deutomerite cylindrical or barrel

shaped, little wider than protomerite, ending in a broadly rounded or flattened square-cornered extremity. Endocyte very dense and brownish yellow in deutomerite, tan in protomerite. Epimerite a small, spherical, hyaline knob. Cysts yellow-orange, 300μ in average diameter, spore ducts short, ten or more in number. Spores extruded in chains, barrel shaped, $5 \times 8\mu$.

Taken at Lincoln, Neb., Colorado Springs, Colo., and Urbana, Ill. Hosts: *Melanoplus femur-rubrum* (deGeer); *M. differentialis* (Uhler); *M. coloradensis* (?); *Encoptolophus sordidis* (Burm.); *Schistocerca americana* Burm.; *Melanoplus bivitattus* (Say); and *Hesperotettix pratensis* Scudder. Habitat: intestine and pyloric caeca.

This species was first described by Hall (1907) as *Hirmocystis rigida*. Crawley (1907) found it shortly after and named the species *Gregarina melanopli*. Ellis (1913) changed the name to *Gregarina rigida*.

Leidyana solitaria n. gen., n. sp. (Fig. 20): Sporonts solitary, cylindrical. Maximum length 500μ , maximum width 160μ . Ratio, length protomerite: total length :: 1:5 to 1:7. Ratio, width protomerite: width deutomerite :: 1:1.3 to 1:1.7. Protonerite conical, dilated in middle portion, constricted deeply at septum. Protonerite slightly wider than high in adults. Deutomerite cylindrical to elongate ellipsoidal, sometimes tapering, rounded posteriorly. Endocyte of protonerite pale tan, translucent, of deutomerite very dense, black in transmitted light, the two parts very plainly demarked, nucleus not visible in vivo, spherical, with one or two small karyosomes. Epimerite a large, globular, hyaline knob on a short, slender stalk. Cysts spherical, 350μ in diameter (including the transparent covering). Dehiscence by spore ducts one to twelve in number. Spores given out in chains, barrel shaped, 3 by 6μ .

Taken at Cold Spring Harbor and Oyster Bay, L. I., N. Y., Haverford, Pa., and Urbana, Ill. Host: *Gryllus pennsylvanicus* Burm. Habitat: intestine.

This species was described by Crawley (1907) under the name *Stenophora erratica*. The mode of cyst dehiscence, however, precludes the possibility of its belonging to the family Stenophoridae. I have placed it in a new genus under the family Gregarinidae, characterized as follows: *Leidyana* n. gen. Sporonts solitary, epimerite a simple globular knob, dehiscence by spore ducts, spores doliform.

I should restrict the genus *Gregarina* to biassociative sporonts only, the other characters being identical with those of the new genus.

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EXPLANATION OF PLATES 1 AND 2

Fig. 1.—Sporont of *Stenophora diplocorpa* n. sp. from camera lucida drawing of the original.

Fig. 2.—Sporont of *Stenophora impressa* n. sp.

Fig. 3.—*Stenophora lactaria* n. sp.

Fig. 4.—*Amphoroides calverti* (Crawley).

Fig. 5.—*Gregarina katherina* n. sp.

Fig. 6.—*Gregarina barbarara* n. sp.

Fig. 7.—*Gregarina globosa* n. sp.

Fig. 8.—*Gregarina monarchia* n. sp.

Fig. 9.—*Gregarina intestinalis* n. sp.

Fig. 10.—*Gregarina gracilis* n. sp.

Fig. 11.—*Gregarina tenebrionella* n. sp.

Fig. 12.—*Gregarina fragilis* n. sp.

Fig. 13.—*Steinina rotunda* n. sp., a trophozoite with epimerite

Fig. 14.—*Gregarina nigra* n. sp.

Fig. 15.—*Gregarina stygia* n. sp.

Fig. 16.—*Gregarina galliveri* n. sp.

Fig. 17.—*Gregarina illinensis* n. sp.

Fig. 18.—*Gregarina achetae-abbreviatae* Leidy.

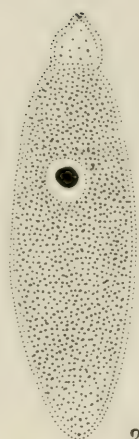
Fig. 19.—*Gregarina rigida* (Hall) Ellis.

Fig. 20.—*Leidyana solitaria* n. gen., n. sp.

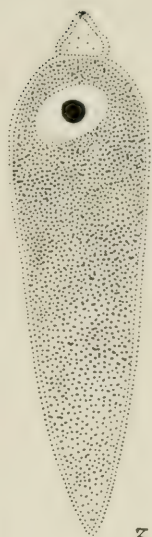
PLATE 1



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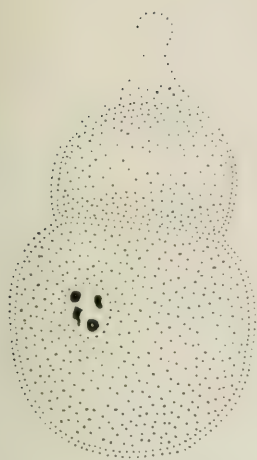


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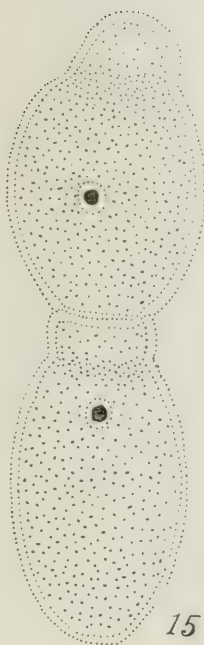
PLATE 2



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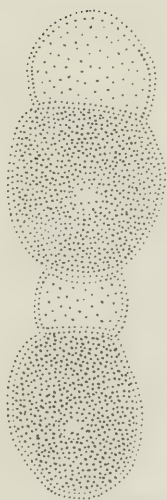
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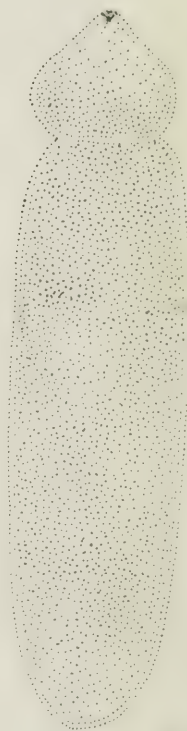
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PNEUMONYSSUS FOXI, NOV. SP.

AN ARACHNOID PARASITIC IN THE LUNG OF
A MONKEY (MACACUS RHEBUS)

FRED D. WEIDMAN

On March 7, 1914, an adult male *Macacus rhesus* died in the Philadelphia Zoological Gardens with a subacute catarrhal colitis. Its lungs contained, in addition, sixteen to twenty small lesions about equally divided between the two organs. They were nodular, 2 to 5 mm. in diameter, were situated immediately under the pleura and slightly elevated above the same. The smaller ones were firm throughout, the larger ones with softer umbilicated centers and indurated edges. In some of the lesions smaller hard points were to be made out, suggesting a conglomerate lesion. When fresh they were pink or gray and upon incision found to contain granular gray material with granular gray walls. Upon scraping out the centers of the lesions and examining the contents, the parasitic character of the lesions was at once determined.

Microscopically, the sections of the lesions exhibit sections of an arthropod, lying in granular necrotic material, together with brownish black, extremely finely granular detritus (excrement). Around this there is a slight round-cell infiltrate, very poor in leukocytes and in close relation to a bronchus. A thick fibrous wall of very young type surrounds the whole.

The material used in making the following description was obtained by gently scraping out the interiors of two of the pulmonary lesions. This yielded, from one sac, eleven females and one male; from the other, five females. Free larvae or ova were not found. The parasites had been fixed in situ by formaldehyd 4 per cent., followed by washing in water and hardening in alcohol. Some specimens were teased, the remainder preserved entire and examined first in a watery medium, followed by clearing in glycerin-alcohol mixture, or Farrant's medium. During these studies it was found that the delicate membranes of the caroncle could only be satisfactorily examined *prior* to prolonged immersion in glycerin or Farrant's medium, both of which had a marked tendency to produce shrinkage of the same. Finer structures, such as hairs and plates, were only successfully determined after clearing for several days and examination with the oil immersion lens. All of the specimens proved to be

clearly of the same species. None were observed living, the material having been submitted subsequent to fixation.

Grossly, they were barely visible as minute, ovoid, glistening, opaque, white or faintly yellowish bodies.

THE FEMALE

Average females, unruptured and not much distorted or flattened by pressure measure as follows:—

Pubescent female	0.850	×	0.450	mm.
Ovigerous female	0.960	×	0.560	mm.
	0.940	×	0.500	mm.
	0.750	×	0.400	mm.

This gives an average of 0.875×0.478 mm. They are ovoid, the body not divided or constricted, the greatest width lying immediately behind the last pair of legs. In none of the specimens, and this also applies to the male, can the outline or even site of internal organs be made out. At most the ovum is discoverable, with occasionally the outlines of a folded embryo, in a ruptured specimen.

In one of the teased specimens dorsal and ventral plates of muscle can be made out. Each lies median, between the two middle pairs of legs. The ventral is much the heavier. It sends many fasciculi laterally to the coxae, a few radiate anteriorly to the capitulum and several posteriorly to the ends of a long, special muscle band extending transversely between the last pair of coxae. Fasciculi also radiate from the dorsal plate, a few anteriorly and many laterally to the coxae. There are other bands extending circumferentially around the posterior body half, but the condition of the specimen illustrating the muscular arrangement does not permit exact description here.

The head is continuous with the body both dorsally and ventrally. There are no eyes. The rostrum projects slightly beyond the general body contour, is triangular, the apex rounded, the lateral edges curled dorsally, but curving quickly ventrally again at the apex. In this way a short, broad longitudinal groove is produced over the dorsum of the rostrum. The hypostome is quadrilateral, save for a slight median anterior marginal peak, a little longer than broad and does not project beyond the rostrum. Its surface is finely pebbled like morocco leather. A shallow longitudinal median furrow extends through almost its whole length, stopping just short of the anterior margin. In the depths of this groove 8 to 13 short blunt teeth are noted, set in oblique manner with their points directed anteriorly and ventrally. On either side of the furrow transverse or slightly oblique muscle bundles are seen under the cuticle, which appear to pass anteriorly and median to the bases of the teeth. Midway between the furrow and the lateral hypostomal border, and well short of the

anterior margin of the hypostome, a hair is seen on each side. In addition two small papillae are present on each side of the furrow on the anterior margin of the hypostome.

The mouth parts are markedly retracted, barely projecting beyond the hypostome and not at all beyond the rostrum. The palpi lie dorsal and lateral to the mandibles. They consist apparently of three segments, of which the terminal is best seen, subspherical and capped by a stout, moderately long hair. The mandibles are chelate, lie within a sheath in which they are so far retracted as to be generally invisible. At times the two pointed, untoothed fingers of each, one shorter than the other, may barely extend beyond the mouth parts.

There are four pairs of legs, the first two geniculate. The first pair is close to, and its proximal segment (coxa) fused with, the capitulum. The second pair is close to the first pair, with no intercoxal space. The third pair is but a short distance, perhaps the width of a coxa, behind the second, and the fourth pair is at a similar distance from the third. There is no special interval separating the first and last two pairs of legs. The first three legs are subequal in length (0.22 mm.), the last one a little longer (0.25 mm.).* No legs are as long as the body width. Each leg has six articles, although at first glance they may appear to have seven or eight. This is due to the presence of the "fehlenden" muscular insertion of Winkler, which produces a ring simulating the division line between two segments. This ring appears constantly in the tarsus, is well marked constantly in the femora of the two middle pairs of legs and more faintly and only fairly constantly in the femora of the first and fourth pairs of legs. The tibia and patella are of about the same size, much shorter and heavier than the tarsus. The femur is at least twice as long as broad. The trochanter and coxa are much heavier than any of the other segments and irregularly pyramidal in form. The coxa is fully twice as broad as long, its posterior wall long, its anterior shortened by half in the last pair of legs and almost to nil in the three anterior pairs. Its ventral surface bears, distally, a cuneiform process which bears against a special chitinous plate of the trochanter. The cuticle over all segments bears long, stiff, straight or slightly curved hairs, their insertions surrounded by a low rounded ridge. They are most numerous on the distal segments and scarce but constant on the proximal ones. Spurs are constant on the three distal segments, on the other three variable.

* An ovigerous female 0.75×0.40 mm. is used for all measurements unless specially noted.

As a rule there are none on the coxae or trochanter; if present at all it is generally the first two pairs of legs which bear them.

Each leg has a special terminus. The first pair has two arched, subparallel, fairly heavy dorsal claws. Their extremities overhang or touch a single, median, compound-curved, ventral projection with a sharp point, which has the character of neither claw nor spine, appearing more like a chitinous, elongated, pointed tongue. This leg has no caroncle, the base of the claws being set directly into the substance of the tarsus.

The second pair is terminated by a short, broad caroncle measuring about twice as long as broad. It is pyriform, the handle inserted into the tarsus, the ventral and lateral distal parts open to permit protrusion of the claws, the whole now coming to resemble a hood with the opening directed ventrally. It is delicately membranous distally, and heavier proximally. Two strong claws are attached to its dorsum internally. These are parallel at their origins deep in the caroncle, but at their middles become strongly bent laterally, their tips thus coming to diverge and often to project laterally beyond the margin of the caroncle. The details in the depths of the caroncle (the handle) are uncertain. It appears that a median ventral, blunt, chitinous tooth extends from it parallel to the ventral caroncular wall and perhaps continuous with it. It may be straight or lightly curved. The margin of the proximal border of the opening shows a short, pointed, median projection.

The third pair of legs is terminated by a double hooked caroncle precisely like that of the second pair.

The fourth pair also has hooked caroncles built on the same general plan as the preceding, but they are much more slender, measuring about three times as long as broad. Its hooks, too, are much more delicate, more gracefully curved and do not, in the specimens studied, extend beyond the cavity. They are supplemented by a smaller, straighter pair deep in the caroncle, which is not always visible, probably from retraction. The median tongue or tooth is again seen here, but is much smaller, and, too, the median, marginal peak is seen at the proximal border of the caroncular opening.

The cuticle appears for the most part to be soft. It has a pebbled appearance, the elevations so low, far apart and of such irregular size (but always small) that its roughness is not at first sight apparent. In special locations it has the appearance mentioned when describing the hypostome, namely, like a very fine morocco leather. Here the elevations are very small, of uniform size, closely placed and refractile.

One such special area has already been described over the hypostome. A second lies ventrally, suggesting a sternal plate. It is median and extends from the interval between the first pair of legs to a line between the middle of the third pair. It is about three times as long as broad, subelliptical, with both ends flattened. It is not quite so sharply marked off from the surrounding integument as a plate should be. Just within its lateral margins lie six hairs. Two are directly at the anterior margin. The second pair is a little farther apart and between the second and third coxae. The third pair is separated a distance intermediate between the first and second and lies well anterior to the posterior margin of the area, that is, about opposite the middles of the third coxae. All hairs are directed posteriorly.

A third special area lies dorsally, again resembles a plate, is by far more extensive than the ventral and well marked off from the rest of the integument. It extends from a point immediately behind the rostrum to one a short distance behind the fourth coxae. It is broadly ellipsoidal except that the posterior end is roundly pointed. In its widest part it occupies the middle two fourths of the dorsum. It bears five pairs of hairs. The first pair is at the anterior margin. The second is closer behind and much closer together. The third is close behind the second, but now, again, at the lateral margins, and is the farthest apart of any of the five pairs. This brings it on a line with the posterior border of the second coxae. The interval between the third and fourth pair is about the same as that between the first and third. The fourth lies about the same distance below the fourth as that between the third and fourth pairs. These last two are very close together, being the closest of all the pairs. All of the hairs lie in the anterior two thirds of the area, the narrower posterior third being quite naked. This shield bears many small groups of pits commonly ascribed to the traction of subjacent muscular attachments. These groups are on the whole of linear arrangement, paralleling the scutal border (see plate).

The fourth special area lies around the anus. This orifice is terminal and round. In some specimens it is everted and projects beyond the general body contour. This is doubtless a pressure artefact. The special perianal area is subelliptical, with its longer dimension placed longitudinally. Three equidistant hairs lie at its margins, two lateral ventral and one median dorsal. They arch over the anal orifice. No lateral thorns are seen, although in one specimen the fracture and eversion of the anal margins by pressure gave this appearance.

In addition to the hairs which have been mentioned in connection with special areas, a dorsal pair is occasionally found at the level of the broadest part of the body posteriorly and far apart, lying well lateral to the black lines produced by the intestines. A ventral pair is also at times discoverable posterior to the plane of greatest body width. These last two pairs are not constant. Those of the three special areas are constant. All corporeal hairs are inserted in a special ring similar to the ones mentioned in connection with the hairs on the legs, and all are directed posteriorly.

There is but one pair of stigmal plates. They lie between and slightly dorsal to the third and fourth coxae and are about three times as long as broad, the narrower end directed cephalodorsad, the broader end containing the orifice. They show two or three faint curved transverse lines in imitation of segmentation.

The vulvar orifice lies ventrally in the middle of the transverse bridge joining the fourth coxae. It is longitudinal, fissural, short, and flanked by narrow, linear, chitinous plates. It appears to be continuous above with the posterior angle of a laterally elongated triangular opening whose other two angles extend far laterally along the chitinous bridge.

The intestines are indicated by two deep, black, tortuous lines extending longitudinally close to cuticle dorsally.

THE MALE

The solitary male discovered is adjudged such from its slenderer proportions (it measures 0.55×0.25 mm.), from the presence of a special anterior ventral orifice, and the lack of the vulvar orifice. In most other respects it is identical in external appearance with the female. All legs have the two dorsal hooks and one ventral piece, there is no caroncle on the first, short broad ones on the two middle ones and a longer, slenderer one on the fourth. The chelicerae in this specimen are, by chance, far extended. At most only the tips of the fingers happened to project in the case of the females. As shown in this male, each is projected from a sheath, extending a short distance beyond the hypostome. Each chelicer has a sharply pointed, lateral, longer, and median shorter, finger, both springing from a common base. The lateral one bends mesially and anteriorly, describing a compound curve. The median one curves upward.

The genital orifice of the male appears close behind the hypostome as a small circular aperture. From it a tube leads posteriorly for some distance directly under the ventral cuticle in a shelving manner, so that to superficial inspection it appears like a median longitudinal furrow.

THE LARVA

A larval form was found close to a ruptured female within which no ovum could be found, from which it is surmised that it escaped from the latter during technical manipulation. The larva measures 0.550 mm. \times 0.280 mm., is oval and has six legs, folded ventrally and with long hairs extending, tuft-like, from the distal segments. The anal plate is clearly marked and provided with three hairs.

ZOOLOGICAL POSITION

The writer places this parasite in the genus *Pneumonyssus* only tentatively and with much unwillingness. In several respects it does not agree with the generic and, furthermore, the superfamily diagnosis. In the former there should be no shields, in the latter no hypostomal armature. Following Banks' key, however, there is no alternative. It is felt that the time for a rearrangement of these endoparasitic Acarians is at hand, and in the expectation that this will be done in the near future it is deemed inadvisable to attempt to announce a new genus for this one species. It should be pointed out at this time, however, that this is the first time that a male *Pneumonyssus* has been described, and that the position of its sexual orifice places it close to, if not in, the Gamasidae. Indeed Banks has already hinted, in a personal communication, that the genus *Pneumonyssus* may belong more properly to the Gamasidae than to the Dermanyssidae, on the basis of certain features noted in the nymph of *P. simicola*.

As far as known to the writer, this is the fifth species of arachnoid described from the air passages of a monkey.

Pneumonyssus simicola was found by Banks (1904) in the lungs of a Javanese monkey (*Cyanocephalus* sp. ?) dying of opium poisoning in Java.

Pneumonyssus duttoni was found by Newstead and Todd (1906) in the trachea and bronchi of eleven Schmidt's monkeys (*Cercopithecus schmidtii*) in the Congo.

Pneumonyssus griffithi was found by Newstead (1906) in the lungs of six rhesus macaques killed in England after exposure to tuberculosis.

Pneumotuber macaci was found by Landois and Hoepke (1914) in the lungs of a *Macacus rhesus* killed in Breslau.

The description of no one of these four species agrees with this parasite or permits its inclusion in that species.

Pneumonyssus simicola has "a broad pulvillus beneath the claws in some specimens, probably females," whereas a pulvillus is constant on legs i, ii, and iii in *P. foxi*. *P. simicola* has four small bristles on dorsum, *P. foxi* has ten. With *P. simicola* bristles are not present on the coxae. On *P. foxi* they are commonly present. *P. simicola* has no dorsal plate as has *P. foxi*.

P. duttoni is at once excluded by the presence of a transverse body division, two pairs of stigmal plates and its very elongate form. It has a dorsal shield.

P. griffithi has stiliform mandibles; those of *P. foxi* are chelate. The dorsal shields of the former has six hairs, those of *P. foxi* ten. The arrangement of the groups of pores (pits) here is different, too.

Pneumotuber macaci has no shields, only one claw on dorsum of tarsi i, ii, and iii and a pulvillus on tarsus iv only. There are four dorsal hairs as against ten for *P. foxi*.

Finally, and of most importance, with none of the above species is a ventral plate or the special features of the hypostome mentioned. It is true that these are easily overlooked and may have been present in the other species, but until this is shown the parasite here described must remain a new species, though much needed future rearrangement is likely to place it in a different genus or family.

The technical description of the new species follows:

Class, Arachnoidea; order, Acarina; superfamily, Gamasoidea; diagnosis (Banks): Hypostome small, without teeth; venter without furrows; body often with coriaceous shields; posterior border never crenulate; no eyes. Family, Dermanyssidae; diagnosis (Banks): Parasitic on vertebrates; mandibles fitted for piercing; body sometimes constricted. Genus, *Pneumonyssus*; diagnosis: * A Dermanyssid; stigmal plate a little more than twice as long as broad, situated above and between the coxae of the third and fourth pairs of legs. Body without apparent shields; mouth parts retracted in the head, the palpi very short, scarcely visible; the mandibles have apparently both fingers very slender, elongate and pointed, probably used for pricking the tissues. The legs are stout and short, subequal in length, none as long as width of body, each terminated in two subequal claws. Body nearly twice as long as broad, in the male more slender. Legs with stiff bristles, but body nearly destitute of hairs.

Type species, *P. simicola* Banks.

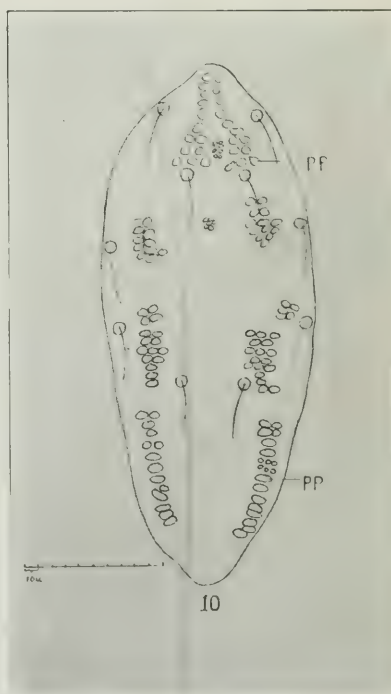
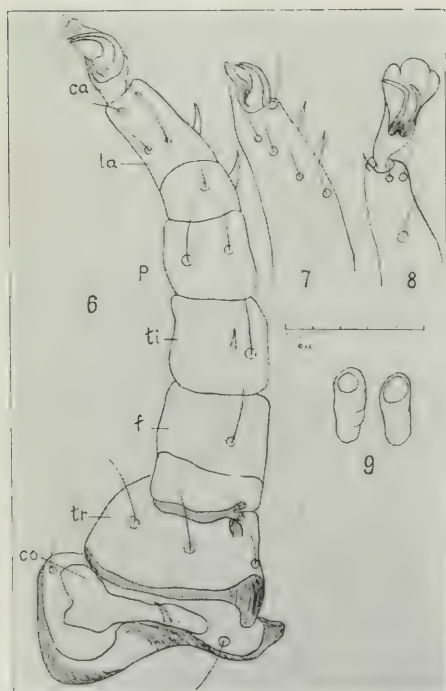
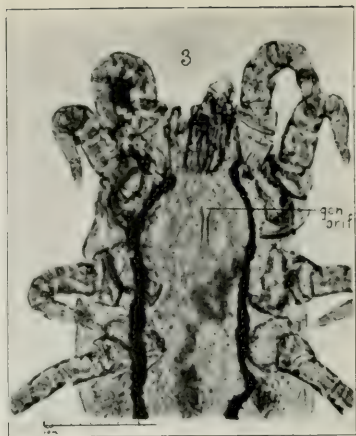
P. foxi † n. sp.; diagnosis: Adult females, yellowish white, opaque, in width a little more than half the body length. Dorsal shield with ten hairs and pitted areas, ventral with six hairs. Anal plate present with three hairs. All tarsi furnished with two dorsal claws, all except leg i with caroncle in addition; all articles hirsute and most also spinulose. Both tarsi and femora subdivided.

* Kindly furnished, together with other information, in a personal communication by Dr. Nathan Banks.

† Dedicated to Dr. Herbert Fox, who performed the autopsy upon the animal, recognized the parasitic nature of the lesions and submitted all the material to the writer for identification.



PLATE 1



Palpi of three segments, all short, the terminal one capped by a short bristle. Mandibles chelate in both sexes. One pair of stigmal plates between and dorsal to coxae iii and iv. Hypostome bears a median longitudinal row of 9 to 13 teeth, carries ten hairs anterolaterally and four anterior marginal papillae. Vulva short, median and fissural at level of coxae iv. Adult males measure a little less than half as wide as long. Sexual orifice circular and close behind capitulum. Larva hexapod, oval, 0.55×0.28 mm., bears anal plate. Length 0.875 mm., breadth 0.478 mm.

Habitat, lungs of monkey (*Macacus rhesus*).

Autopsy number P. Z. G. 3156.

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EXPLANATION OF PLATE

Fig. 1.—Ovigerous female viewed ventrally. Magnification about 66 times.

Fig. 2.—Adult (?) male viewed ventrally. Magnification about 66 times.

Fig. 3.—Same as Figure 2 but more highly magnified. Shows mouth parts and ventral shield, the latter with genital orifice and six hairs. Magnification about 240 times.

Fig. 4.—Teased adult female: *ds*, dorsal shield; *vs*, ventral shield; *mf*, muscle fasciculi. Magnification about 100 times.

Fig. 5.—Dorsal shield from Figure 4 more highly magnified; *pp*, pits; *mf*, muscle fasciculi. Magnification about 260 times.

Fig. 6.—Leg ii and iii: *ca*, caroncle; *ta*, tarsus; *p*, patella; *ti*, tibia; *f*, femur; *tr*, trochanter; *co*, coxa.

Fig. 7.—Extremity of Leg i.

Fig. 8.—Extremity of Leg iv.

Fig. 9.—Stigmal plates.

Fig. 10.—Dorsal shield. Compare with Figure 5.

CESTODE CYSTS FROM MUSKRAT

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The material was collected from a muskrat found near Washington, Pa., on Feb. 8, 1884. Four cysts were found, three embedded in the liver, and one in the peritoneum. The cysts were elliptical in outline, the largest measuring 13 by 9 mm. When opened, the contained cysticercus was seen to have developed into a strobila, with the bladder portion reduced to a small, flattened, spatulate body with collapsed walls.

The strobiles were milk-white and actively contractile. When first released they showed a tendency to thicken anteriorly so that the whole strobila became more or less clavate. Two of them, measured immediately after removal from the cysts, were 121 and 143 mm. respectively. After lying in water over night the larger specimen measured 212 mm. A few hours later it measured 300 mm. The



Fig. 1.—Pair of hooks. Length of longer hook 0.4 mm.

anterior end, for a distance of about 175 mm. was about 6 mm. in breadth and 3 mm. in thickness. The posterior third narrowed uniformly to 2 mm. The remnant of the bladder at the posterior end was 6.5 mm. in length and 5 mm. in breadth. When this specimen was placed in alcohol at the end of forty-eight hours it measured 325 mm.

The diameter of the scolex in a mounted specimen is 1.06 mm. The suckers are rather prominent and directed anteriorly. The portion of the scolex in front of the suckers, when the hooks are completely everted, is conical-truncate. There are two circles of hooks, those in the anterior circle being the larger. The hooks lie in pairs, a pair consisting of a large and a small hook (Fig. 1). The number of hooks, estimated from a study of living specimens, seen lateral view, was fourteen in each circle. Another specimen seen in

front view had eighteen hooks in each circle. The hooks of the anterior circle are about 0.45, and those of the posterior circle 0.26 mm. long.

Proglottids begin a very short distance back of the suckers where they are about 0.5 mm. in length. In the larger alcoholic specimen the proglottids toward the middle of the length are 5 mm. in breadth and 0.72 mm. in length; farther back the breadth is 4 mm. and the length 0.8 mm.; toward the posterior end the breadth is 3 mm. and the length 0.56 mm. Sinuous marginal vessels are visible in the stained and mounted segments, but no rudiments of genitalia were seen.

The size and shape of the hooks and the appearance of the bladder-worm indicate that this cestode is the form known as *Cysticercus fasciolaris* the larval stage of *Taenia crassicollis*.

SARCOPHAGID LARVAE FROM THE PAINTED TURTLE

F. E. CHIDESTER

Rutgers College, New Brunswick, N. J.

In studying the blood of vertebrates in my course in histology during the past winter, I used a specimen of *Chrysemys picta*, the common painted turtle. In pulling the right hind leg back against the plastron before making the stab for blood, I noted that a hardened cylinder partly protruded from the thigh. When the object was completely extruded, it parted in the middle and five sarcophagid larvae were discovered.

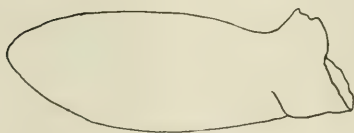


Figure 1



Figure 2

Fig. 1.—Horny case containing Sarcophagids.

Fig. 2.—Sarcophagid larva from the turtle. (Ventral aspect.)

The specimens were submitted to Dr. T. J. Headlee and to Dr. L. O. Howard. For information regarding the larvae, I am indebted to them and to Mr. C. H. Richardson, assistant state entomologist of New Jersey.

So-called bot-fly larvae have been reported by Packard (1882), True (1884), and Wheeler (1890). Wheeler corrected the previous writers and placed the larvae among the Sarcophagae. Most recently Kepner (1912) has described in detail larvae undoubtedly of the same species as my own specimens. There is no question that the tortoises are infested by sarcophagids and the reason for this brief note is found in the peculiar modification of the epidermis of the host which formed a case for the larvae.

This horny epidermal case (Fig. 1) was 15 mm. long, 5.5 mm. broad, and from 0.5 to 0.6 mm. thick. The outside was regular and smooth except at one point, where it was slightly indented. The

mouth of the case was irregular and before being disturbed the object was scarcely discernible on the skin of the turtle. The hind leg was measured, and the thigh proved to be only 25 mm. in length and 13 mm. in diameter. The leg was completely paralyzed, and although the specimen was kept alive for a month after the case had been removed, use of the leg was not regained.

The larvae (Fig. 2) were kept alive for some days, then two were placed in moist earth in an attempt to secure pupation. The results were negative, however. The other specimens were turned over to the entomology department and one of them was sent to the department of agriculture for identification. So far as the writer knows no imagoes other than the four females secured by Wheeler have been bred from the turtle-infesting sarcophagid.

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NOTES

The life-history of the human blood fluke is undoubtedly one of the most important of unsolved problems in parasitology. For many years, in fact ever since the discovery of the adult parasite in Egypt by Bilharz in 1852, this question has commanded the attention of able investigators, but practically no positive results have followed their work. Local tradition supported by circumstantial evidence led to general acceptance of the view that infection with the African species, *Schistosoma haematobium*, was acquired by bathing in infected waters. After extended study in regions of pronounced infection Looss concluded that infection took place directly through the skin and that the infecting stage was the miracidium, which underwent metamorphosis in the body of the final host, probably in the liver.

The discovery of a new human species (*Schistosoma japonicum*), was augmented by its later demonstrated occurrence in various small mammals and experimentation became possible. Utilizing this opportunity, Leiper and Atkinson recently made a trip to the East and as a result of their work, which was unfortunately cut short by the war, have published* most important studies on the life history of this fluke.

After a search lasting nearly three months and a river journey of a thousand miles, they secured a dog so heavily infected that the evacuations consisted of mucus and blood crowded with eggs. The local mollusks were placed in water swarming with miracidia and were watched to detect those species which exercised a pronounced attraction for the free-swimming fluke embryos. A small brown snail of a new genus, *Katayama nosophora*, displayed such an attraction, "The small dark head and foot speedily became festooned with little white specks and it was obvious from the agitated manner in which the snail repeatedly attempted to brush them off that their presence was a cause of considerable irritation."

In the liver of this snail were sporocysts containing cercariae with bifid tails. The cercaria had a short bifurcated gut with no trace of a pharynx. Laboratory-bred mice were exposed to infection in water containing free cercariae from the teased snail liver. At Aden on the home voyage the few mollusks living were sacrificed, and the last mouse was exposed to infection. When examined in London a month later this mouse contained live male and female blood flukes in copula in the portal vessels. These factors show conclusively that this schistosome has a life-cycle like that of the digenetic trematodes.

The cercaria is covered by minute spines, the oral sucker is enormous, equal to about one-third the length of the body, and urn shaped. Between the lateral branches of the intestine are several masses on each side, the undeveloped sex glands. The snail which functioned as secondary host though abundant proved to be entirely new.

* Brit. Med. Jour., January, 1915; China Med. Jour., May, 1915.





PROFESSOR STANISLAUS VON PROWAZEK

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PROFESSOR VON PROWAZEK *

(WITH PORTRAIT)

Im Dienste der Wissenschaft und des Vaterlandes starb am 17. Februar Professor Stanislaus von Prowazek, der Leiter des Protozoenlaboratoriums am *Institut für Schiffs- und Tropenkrankheiten* in Cottbus an Flecktyphus. In ihm verliert nicht nur das Tropeninstitut sein bedeutendstes Mitglied, sondern die gesamte wissenschaftliche Welt einen Forscher von universeller Bedeutung.

Prof. Dr. v. Prowazek war 1875 in Oesterreich geboren. Er studierte in Prag und Wien. Nachdem er kurze Zeit Assistent am Institut für experimentelle Therapie in Frankfurt a.M. (Direktor Geh. Rat Ehrlich) und am zoologischen Institut der Universität München (Prof. Hertwig) gewesen war, wurde er 1903 auf Veranlassung von Fritz Schaudinn, dem damaligen Vorsteher der Abteilung für Protozoenforschung des Kaiserlichen Gesundheitsamtes dorthin nach Berlin berufen und wurde, als Schaudinn dem Rufe ans tropenhygienische Institut zu Hamburg folgte, der provisorische Leiter des Protozoenlaboratoriums im Kaiserlichen Gesundheitsamt und nach dem allzu frühen Tod des genialen Schaudinn sein Nachfolger in Hamburg (1907). Er war mit Schaudinn aufs innigste befreundet und hat nicht nur die weitausblickenden Gedanken dieses Forschers nach seinem Tode aufs glücklichste weiter verfolgt, sondern auch die Wissenschaft mit vielen, glänzenden, selbständigen Gedanken und Beobachtungen bereichert. Seine Studien über die Physiologie und Biologie der Zelle und der Protozoen im besonderen, seine Untersuchungen über Variola und Vakzine, über Trachom, Blennorrhoe und andere Augenkrankheiten und die darauf gegründete Erfassung der als Infektionserreger weit verbreiteten Chlamydozoengruppe (Pocken, Hundswut, verschiedene Augenkrankheiten und Tropenkrankheiten) machten ihn zur bedeutendsten Autorität unter den Forschern auf dem Gebiete der modernen Protistenkunde.

Kurz nach dem Tode Schaudinns hatte er die Ausreise nach Nieder-

*This sketch was written for THE JOURNAL on request by Prof. W. Michaelsen of the Naturhistorisches Museum in Hamburg.

ländisch-Indien als Mitglied der Neisser'schen Syphilis-Expedition angetreten, gelegentlich derer er mit Halberstädter seine Entdeckungen über die Aetiologie des Trachoms machen konnte; nach seiner Rückkehr trat er die Stelle am Tropeninstitut am 16. Juni 1907 an.

Hier am Institut entfaltete er eine überaus fruchtbare Tätigkeit, teils in stiller Laboratoriumsarbeit, teils auf ausgedehnten Forschungsreisen. Von diesen führte ihn eine vom 10. Juni 1908 bis 26. Februar 1909 an das Instituto Oswaldo Cruz nach Rio de Janeiro, wohin er gemeinsam mit Prof. Giemsa auf Einladung des brasilianischen Staates beurlaubt wurde zu Lehr- und Forschungszwecken. Von Mitte 1910 bis Ende 1912 machte er gemeinsam mit dem Ophthalmologen Dr. Leber mit Mitteln des Reichskolonialamts und des Hamburgischen Staates eine Expedition nach Sumatra und dem deutschen Südseegebiet zum Studium der Granulose und anderer Krankheiten. Die Früchte dieser Expedition waren nicht nur auf seinem Spezialgebiet reichlich; sondern er hat auch in seinem Buche "Die deutschen Marianen" dank seinem universellen Wissen die Geschichte, Ethnographie, Fauna, Flora und das Medizinische in überaus gewissenhafter und poetisch-reizvoller Form veröffentlicht. Spätere Reisen führten ihn zum Studium des Flecktyphus im Sommer 1913 gemeinsam mit Hegler nach Serbien und im Sommer 1914 nach Konstantinopel mit Rocha-Lima. Mit letzterem wurde er, als im Dezember 1914 im Russenlager in Cottbus eine grosse Fleckfieberepidemie ausbrach, vom Kriegsministerium mit wissenschaftlichen Untersuchungen daselbst betraut. Dort ist er in der ersten Februarwoche 1915 an der Seuche erkrankt und der Infektion am 17. Februar erlegen.

Prowazek war ein Forscher von ungewöhnlich vielseitigem Wissen; er beherrschte nicht nur meisterhaft das Gebiet der Zoologie und ihre Grenzgebiete, sondern besass auch auf dem Gebiete der Botanik, Physik, Chemie, und Philosophie ungemeine Kenntnisse, ebenso auch auf medizinischem Gebiet, besonders der Immunitätslehre. Dies zeigt sich in seinen zahlreichen Arbeiten auf diesen Gebieten ausgeprägt. Prowazek war ungeheuer fleissig und seine Arbeiten zeichnen sich durch eine erschöpfende Gründlichkeit aus. Für den Mediziner sind vor allem bedeutungsvoll geworden seine Studien über die Biologie und Physiologie der Zelle und der Protozoen im speziellen. Sein Buch "Die Physiologie der Einzelligen (Protisten)" enthält eine Fülle von ihm gefundener neuer Tatsachen und die Erörterung neuer Probleme. Vor allem die Tropenmedizin verdankt Prowazek als Lehrer und Forscher sehr viel; viele unserer Tropenärzte, die ihm als Schüler und Freunde näher getreten waren, standen dauernd mit ihm in regem wissenschaftlichem Gedankenaustausch.

Am Tropeninstitut hat er auch eine ausgedehnte Lehrtätigkeit entwickelt und Schüler aus aller Welt fesselte er an sich. Mit Schülern und Freunden als Mitarbeitern hat er auch das gross angelegte "Handbuch der pathogenen Protozoen" herausgegeben, dessen Abschlussband, für den von ihm selbst zahlreiche fertige Manuskripte vorliegen, er nicht mehr erleben konnte. Das von Schaudinn begründete "Archiv für Protistenkunde" hat er gemeinsam mit Hartmann weitergeführt und zur ersten grössten, internationalen Zeitschrift auf diesem Gebiete gestaltet.

Prowazek war ein Mensch, der wenig gern in die Oeffentlichkeit trat, still und zurückgezogen lebte, eine echte Gelehrtennatur. Wer aber das Glück hatte, ihm näher treten zu dürfen, lernte ihn als Menschen von seltenem Wissen und feinsten Kultur kennen, begabt mit viel Sinn für alles Schöne in Kunst und Natur. Im Kreise seiner Tätigkeit war er verehrt und geschätzt von allen, bot stets eine Fülle von Anregungen. So ist sein Tod nicht nur ein unersetzlicher Verlust für die Wissenschaft, sondern auch für seine vielen Freunde.

FURTHER NOTE UPON COMPARISON OF *ENDAMOEBA*
GINGIVALIS (GROS) AND *ENDAMOEBA*
HISTOLYTICA SCHAUDINN

ALLEN J. SMITH AND M. T. BARRETT

From the McManes Pathological Laboratories, School of Medicine,
University of Pennsylvania

In a recent number of this Journal¹ the writers, after an analysis of the records of discovery of parasitic amebae in the human mouth concluded that the oral endamebae of man are referable to two species: *Endamoeba gingivalis* (Gros 1849) and *Endamoeba pyogenes* (Verdun and Bruyant 1907). In an attempt to compare these oral parasites with other parasitic amebae of man the writers suggested that the second species named may be identical with an organism, characterized like it by a large nucleus containing a large, granular, richly chromatinized binnenkörper, found by Ribbert in the ducts of the parotid gland, and believed by the writers to be the same as found some years ago by Ribbert in the renal tubules of a syphilitic new-born infant, by Jessionek and Kiolemengolou in the kidneys, liver, and lungs of an aborted syphilitic fetus, and by Smith and Weidman in the kidneys, lungs, and liver of a non-syphilitic new-born infant and in the lungs of a syphilitic infant one month old, to which these last writers have applied the name of *Endamoeba mortinatalium*. (For literature cf. original article in this Journal.¹) In comparing the first named species of oral endamebae with *Endamoeba histolytica* Schaudinn, the writers asserted so close a morphological similarity that, while unwilling to declare the biological identity of these parasites, they felt unable by microscopic examination alone to differentiate between them. Emphasis was laid in a footnote, added to the paper after its presentation at the Christmas convocation of 1914 before the Association of American Bacteriologists and forwarded with the copy for publication, that this statement had reference to *Endamoeba histolytica* solely in its *histolytica* phase (not the *tetragena* phase); and the same footnote contained a brief record of failures of feeding experiments, thus adding reason for believing in the duality of the species altogether apart from the morphological similarity presented. Unfortunately this footnote was omitted in printing; and through oversight an editorial note, written following a discussion by correspondence, was inserted, emphasizing the *tetragena* phase of *Endamoeba histolytica* as a basis of differentiation and urging the known

1. Jour. of Parasitol., June, 1915, vol. 1, pp. 159-174.

reproductive encystment in this latter phase as a point in separation. The writers have felt that thereby their position has been the more opened to misconception, and through the kindness of the editor are publishing the present note to amend and define their original statement, and at the same time to record briefly their attempts to induce colonic infestation by oral endamebae.

In the *histolytica* stage of *Endamoeba histolytica* Schaudinn the parasite is known to divide by fission and, many at least believe, also by gemmation, but there is no reproductive encystment. In the *tetragena* stage of the same parasite the true encystment with four offspring occurs. In the *histolytica* stage the nucleus is practically invisible in the unstained state; in the *tetragena* stage it becomes visible unstained, and when stained shows a thicker nuclear membrane, a larger binnenkörper and a higher chromatinization. *Endamoeba gingivalis* simulates the first of these phases in its nuclear characters and in its apparent modes of reproduction. It is of course to be expected that sometime and somewhere reproductive encystment does take place; and it is not impossible that in some other situation than in the gums a phase is assumed comparable to the *tetragena* phase of the dysenteric organism, in which reproduction in encystment occurs. We say "elsewhere than in the gums" because we have not noted examples suggesting such change in the pyorrhea material from the many individuals whose parasites we have seen; and a large proportion of these cases was decidedly chronic (the element of chronicity apparently being important to the assumption of *tetragena* characteristics and to encystment reproduction in case of *Endamoeba histolytica*). As far as the question of gemmation is concerned doubt of course is natural. But the writers are not satisfied that the separation of gemmules is merely a phenomenon of degeneration. We have repeatedly seen the throwing off of gemmules by actively moving and apparently normal amebae, and especially in our attempts to cultivate the oral endamebae *in vitro* have found what we believed to be such gemmules in motion, and in stained preparations of the same material similar small protoplasmic bodies containing a minute bit of chromatin. We cannot, of course, declare the fate of these separated particles, but the appearances observed certainly make us unwilling to accept unhesitatingly the view of their inability to grow into adult amebae.

From the morphological similarities of *Endamoeba gingivalis* and the *histolytica* phase of *Endamoeba histolytica* and the occurrence of binary fission and gemmation as modes of reproduction of both (with no reference to the *tetragena* phase of the latter and its known mode of encystment reproduction), the writers held that methods of differentiation other than by comparison of morphological features are essential for differentiation. Since the presentation of the original paper we have

accumulated negative evidence indicating the duality of these oral endamebae and the dysenteric parasites, in the constant failure of attempts to infest the colon with pyorrhea material rich in *Endamoeba gingivalis* Gros. In these experiments, in which we were joined by Dr. Baldwin H. Lücke, assistant instructor of pathology in this laboratory, pyorrhea material bearing active vegetative endamebae was given to two kittens by feeding, to two puppies and two kittens by high rectal enemata, and to four kittens by injection into the colon after laparotomy. In all cases we failed to find amebae in the dejections and to note any evidence of dysenteric symptoms, and in all but two kittens to meet at autopsy with lesions of the colon at all suggestive of success. In these last a few ulcers were met, but smears made from the surface of the ulcers, and serial sections of the lesions, failed to show the presence of amebae. Such failures are not infrequent, it is true, when material known to contain *Endamoeba histolytica* is employed; and a single positive result would outweigh the negative results of our attempts. But because of the uniformity of failure of our experiments we feel that the original impression of biological difference between the species must be maintained in spite of the morphological similarities presented. With such a belief we are disposed to say in spite of the possibility of complete morphological similarity of individual examples of *Endamoeba gingivalis* (Gros) on the one hand and of the *histolytica* type of *Endamoeba histolytica* Schaudinn on the other, that one should in general find that individuals of *gingivalis* are slightly smaller, somewhat less active, with pseudopods commonly more lobose, with a nucleus more frequently central in position, and with a more actively hemolytic capacity (more rapidly destroying englobed erythrocytes and therefore ordinarily showing fewer red cells in the body of the parasite) than will be the case with individuals of *Endamoeba histolytica* in the *histolytica* phase and that intestinal infestation by *gingivalis* is probably impossible. We accept without hesitation the existence of definite differentiating morphological features to separate the oral parasite from the *tetragena* phase of *Endamoeba histolytica*.

NOTES ON THE TREMATODE GENUS TELORCHIS WITH DESCRIPTIONS OF NEW SPECIES*

HORACE W. STUNKARD

In 1889 Lühe created a new genus, *Telorchis*, to contain certain reptilian distome parasites, and designated *D. clava* Diesing (1850) as the type species. In the genus he included *D. poirieri* Stoss. (= *D. gelatinosum* Poirier nec. Rud.), *D. linstowi* Stoss. (= *Monostomum aculeatum* v. Linst.), *D. ercolanii* Montic. (= *D. signatum* Ercol. nec. Duj.), *D. nematoides* Mühl., *D. bifurcum* Braun, *D. pleroticum* Braun, and tentatively *D. arrectum* Mol. nec. Duj. His characterization of the genus states that the testes lie behind one another at the posterior end of the body; the cirrus sac opens somewhat left of the acetabulum and is very long; the ovary is immediately behind the posterior end of the cirrus sac and is separated from the testes by the coils of the well developed uterus; while the vitellaria consist of numerous follicles occupying the space at the sides of the body and approaching more or less closely the anterior and posterior ends. The diverticula of the intestine reach almost to the posterior end of the body; and with the exception of *D. poirieri* all species are armed with spines at the cephalic extremity of the worm. The excretory vessel is long and branches anteriorly in the form of a Y. The oral sucker is usually slightly larger than the acetabulum, though in *D. ercolanii* of the same size.

This same group of reptile distomes was separated by Looss (1899) independently, and also called *Telorchis*, but his article appeared after that of Lühe. Looss selected *D. linstowi* as the type species.

Because of the differences existing between *T. clava* and the other members of the genus, Lühe later (1900) created two subgenera: *Telorchis* with *T. clava* as type, and *Cercorchis* with *T. aculeatus* (= *T. linstowi* Stoss.) as the type. The distinguishing features of the sub-genus *Telorchis* are stated as the absence of an esophagus and the lateral extension of the folds of the uterus beyond the diverticula of the intestine where they may be coiled over the ceca in the form of a figure 8. In the sub-genus *Cercorchis* an esophagus is present and the coils of the uterus are confined between the ceca.

My examination of almost a hundred mature individuals of six different species affords evidence that the lateral extension of the uterus varies largely as a result of congestion with eggs. In the same species one finds some specimens in which the coils of the uterus are confined

* Contributions from the Zoological Laboratory of the University of Illinois, under the direction of Henry B. Ward, No. 55.

between the ceca and others in which the uterine folds overlap the diverticula on one or both sides. Certain species in the genus possess a long esophagus, others a short esophagus, and finally in *T. clava* an esophagus is absent. Furthermore the absence of an esophagus is not always associated with an extracecal coiling of the uterus, and vice versa, since in *T. bifurcus* an esophagus is absent and the uterine coils are intracecal, and in *T. corti* an esophagus is present and the uterine folds often overlap the ceca. These facts show that the characters designated by Lühe are not adequate to subdivide the genus, and since the apparent morphological differences of his types are merely extreme variations of characters common to several species, the sub-genera disappear.

Goldberger (1911) described as new species *T. stossichi*, *T. attenuatus* and *T. robustus*, and formulated a key for the identification and separation of the species.

The genus has a wide distribution, species having been reported from Sicily, Sardinia, Italy, France, Germany, Austro-Hungary, Turkey, Brazil, United States and Canada. So far as is known it is confined to reptilian hosts, species occurring in lizards, snakes and turtles.

The trematodes in this genus are elongate, with more or less parallel sides. The region of greatest width is at or anterior to the middle of the body. They range in length from 1.5 to 13 mm. and in width from 0.25 to 1.6 mm. In *T. arrectus* the ratio of width to length is 1:4, in *T. diminutus* it is 1:5, in *T. clava*, *T. bifurcus* and *T. lobosus* it is about 1:7, while in *T. pleroticus* it is 1:18 and in *T. poirieri* it is 1:22. That part of the body between the oral sucker and the acetabulum is much more motile than the post acetabular region, which is essentially a sac containing the reproductive apparatus.

The cuticula is of uniform thickness in any one worm; it varies from 2μ in *T. parvus* and *T. diminutus* to 11μ in *T. attenuatus*, the thicker cuticula being found in the larger species. Cuticular spines occur on the body arranged in a quincunx pattern and around the external openings in concentric circles. They are deeply imbedded in the cuticula which is raised about the base of each spine in a papilla like structure. Largest around the oral sucker, they gradually diminish in size toward the posterior end of the body where they are indistinct or entirely absent. The rows are separated by distances slightly exceeding the length of the spines. In general, the spines vary in size and proximity directly with the size of the worm. In one specimen of *T. robustus* 13 mm. long, the spines near the anterior end are 6.5μ apart and 5.7μ in length, and in another 6 mm. long they are 3.2μ apart and 3μ in length. In specimens of *T. lobosus* they are 2μ long and in rows

2.2μ apart, and in *T. diminutus* they are 1.5μ long and in rows 1.6μ apart. The cuticula turned in at the external openings is not spinous.

The musculature of the body (Fig. A) is light and delicate so that the worms are translucent.

The excretory system is typical for the genus. The pore is situated at the posterior tip of the body and opens from a large, median collecting duct (Fig. B) which extends anteriorly to about the location of the ovary; there it divides into branches that extend antieriad, one on either side of the median line, just mesal to the intestinal diverticula. These branches can be traced almost to the region of the acetabulum where they disappear. In studying living specimens of *T. corti* I have been able to distinguish the flame cells, but their ducts could not be followed.

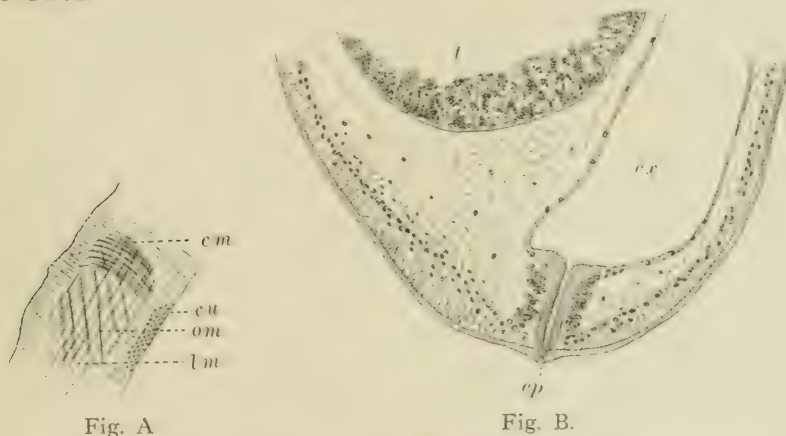


Fig. A

Fig. B.

Fig. A. Tangential section of body wall in *T. lobosus*, showing muscle layers and cuticular spines; *cu*, cuticula; *cm*, circular muscles; *lm*, longitudinal muscles; *om*, oblique muscles.

Fig. B. Sagittal section at median posterior end of body in *T. robustus*; *ep*, excretory pore; *ex*, collecting duct of excretory system; *t*, caudal testis.

The oral sucker is sub-terminal in position, equalling or slightly exceeding the acetabulum in size. The shape of both the oral sucker and acetabulum is subject to considerable variation as can be observed by watching the movements of a living worm. The shape of the sucker at the time of killing and the character of the reagents used influence the shape of the organ in the fixed material, although there seems to be a certain relation between the general shape of the sucker and the species.

A prepharynx is absent in *T. aculeatus* and *T. nematoides* and in some forms can be noted only when the cephalic extremity of the worm is much extended. All degrees of variation in length occur to a distinct

and elongated pouch in *T. bifurcus* and *T. pleroticus*. The pharynx is approximately spherical although either the longitudinal or transverse diameter may be greater. An esophagus is absent in *T. clava* and in *T. pleroticus*, short in *T. aculeatus* and *T. parvus*, and very long in *T. medius* and *T. solivagus*. The ceca meet anteriorly at an acute angle and extend almost to the posterior end of the body, terminating rarely (*T. parvus* and *T. poirieri*) in the inter-testicular zone, and in all other known species behind the caudal testis. Anterior to its bifurcation the digestive tract is lined with cuticula continuous with that of the external surface, and the ceca are lined with digestive epithelium, cells with nuclei close to the fibromuscular wall and cytoplasmic processes extending into the lumen of the canal. If the contents of the ceca are forced caudad the ends may be bulbous or flasklike in appearance, while if the caudal part of the excretory duct is much distended the ceca must necessarily taper gradually.

The testes lie in close proximity, one behind the other near the posterior end of the body, in the median line or slightly to the right and left. In his description of *T. parvus*, Braun (1901) states that due to the flattened condition of the body the collecting duct of the excretory system passes between the testes in the shape of a letter S so that when it is distended it causes the testes to lie obliquely. In most cases the excretory duct is dorsal to the testes and conditions are not those in *T. parvus*. The vasa efferentia pass cephalad, just median to the ceca, the duct from the cephalic testis on the left and that from the caudal testis on the right. The ducts move mediad and dorsad as they pass forward; at the region of the ovary they pass above the excretory tubes and then on the median side of these tubes to the posterior end of the cirrus sac where they empty into the vesicula seminalis. The cirrus sac (Fig. 1) is a long, cylindrical, muscular pouch extending caudad from the genital pore and enclosing the cirrus, vas deferens, prostate, and seminal vesicle. The genital pore is immediately anterior and at the left of the acetabulum. In one specimen of *T. corti* the cephalic testis had divided, the resulting organs lying one on either side of the median line. This specimen was not sexually mature and the vasa efferentia could not be traced.

The ovary is in or near the median line, posterior to the acetabulum, and usually just anterior to the center of the body. The oviduct arises from the dorsal posterior margin and after one or two slight irregularities it enlarges to form the ootype which is surrounded by the large unicellular glands of the shell gland. Figure 7 shows the structures of the female genital system in the vicinity of the ovary. Laurer's canal branches from the dorsal posterior part of the ootype and opens on the dorsal surface; the common vitelline duct enters the ootype just

ventral to and at the left of the origin of Laurer's canal. In some species the proximal end of Laurer's canal is enlarged to form a seminal receptacle and in *T. aculeatus* the enlargement may comprise the entire tube which in one case was filled with chromatin granules, in other instances no enlargement is present, as in *T. robustus*. The uterine tube turns first ventrad and then begins a series of irregular, sinuous convolutions, extending posteriad to the cephalic testis and returning antieriad to the nearly straight metraterm which leads to the common genital sinus. The opening of the metraterm is anterior and at the left of the opening to the cirrus sac. The descending and ascending coils of the uterus occupy separate distinct fields in *T. aculeatus* and *T. parvus*; in *T. bifurcus* they overlap and are in some cases indistinct, while in *T. diminutus* and *T. nematoides* they are so superimposed and confused that only rarely can distinct fields be discerned. In *T. solivagus* Odhner (1902) reports that the descending and ascending limbs of the uterus cross each other to form a figure 8 and other authors mention this crossing or absence of crossing as a specific character, but in *T. corti* both conditions exist.

The vitellaria lie laterad of the ceca, and consist of a large number of follicles, usually arranged in lobes. Typically there are nine lobes on the right and twelve lobes on the left side of the body, although there is considerable variation from this condition. There is a tendency for the lobes to fuse, reducing the number, and the vitellaria of the left side extend farther cephalad and caudad than those of the right. In *T. aculeatus* the lobes are distinct, in most of the species they can be distinguished, while in others (*T. diminutus* and *T. robustus*) the vitelline follicles are not separated into lobes but extend along the sides in an unbroken series. Longitudinal collecting ducts occupy the median face of the vitellaria, and in the region of the ootype short ducts leading mediad from these unite near the median line of the body to form the vitelline receptacle from which the common yolk duct leads to the ootype.

In all sexually mature worms the uterus contains enormous numbers of eggs. They vary in size from 10 by 20 μ in *T. pleroticus* to 22.8 by 40 μ in *T. parvus*, and 21 by 41 μ in *T. diminutus*. It is interesting to note that in the smallest species, *T. parvus* and *T. diminutus*, the eggs are larger than in the largest species, *T. poirieri* and *T. robustus*, where they measure only 14 by 23 μ and 15 by 29 μ , respectively.

If the description given by Stafford (1900) and (1905) of *T. angustus*, and that by Barker and Covey (1911) of *T. leptus* are confirmed by further study, then these species do not belong in the genus *Telorchis* as conceived and discussed in preceding section of this paper. The long distance separating the acetabulum and the genital pore, the dorsolateral location of the latter, and the pre-acetabular position of

the cirrus sac form a complex of such striking and fundamental differences that a natural grouping will remove these forms from the genus *Telorchis*, thereby raising the sub-genus *Protenes* Barker and Covey to generic rank. *Protenes leptus* designated by Barker and Covey must be taken as type.

To Professor Henry B. Ward, under whose direction this work was done, I wish to express my appreciation for criticisms and suggestions.

Telorchis corti sp. nov. (Figs. 1, 4)

Specimens 4 to 7.15 mm. long; 0.35 to 0.5 mm. wide; greatest width at acetabulum, which is 0.14 mm. in diameter, one sixth to one seventh of body length from anterior end. Oral sucker same size as acetabulum; cuticular spines around former 3.4μ in length. Pharynx 70 to 80μ in diameter. Esophagus short, 50μ long, 25μ in diameter. Ovary spherical or slightly oval, in median line or just left of it, about three eighths of body length from anterior end; 0.117 by 0.147 mm. in the smaller specimens and 0.147 by 0.176 mm. in the largest; long axis parallel to that of body. Receptaculum seminis present; Laurer's canal opens just caudad of ovary. Uterus extends posteriad on left side, returning on right, rarely descending and ascending limbs cross about one third of distance from ovary to cephalic testis, forming a figure 8. Coils of uterus occasionally overlap diverticula through central half of distance from ovary to cephalic testis on one or both sides. Metraterm almost straight, extending caudad from genital pore one fourth to one third of distance to ovary. Vitellaria arranged in lobes; separate lobes often not distinct; begin about one-third of distance from ovary to acetabulum, cephalad of posterior end of cirrus sac; extend about five sixths of distance from ovary to cephalic testis. Twenty to forty follicles in each lobe. Testes spherical or oval, about equal in size, 0.2 to 0.29 mm. in length; 0.16 to 0.24 mm. in width; separated by 0.05 to 0.1 mm. Cirrus sac extends caudad from genital pore three fourths of distance to ovary; 1.12 to 1.18 mm. in length; 0.088 mm. in width. Vas deferens much coiled. Mature eggs average 31 by 15μ ; those near ovary broader, measuring 30 by 19μ .

In my material there are many immature specimens. The young worms are proportionately wider than the adults with the region of greatest width at the pharynx. The smallest mounted measured 0.65 mm. in length and 0.16 mm. in width. In this specimen the oral sucker is 0.085 mm. in diameter and the acetabulum is very small, 0.03 mm. in diameter. The esophagus is longer and the ceca are larger proportionately than in the adult. The cirrus sac, ovary and testes appear merely as masses of heavily staining cells. In a specimen 1.7 mm. long the body is 0.2 mm. in width. The suckers have increased in size, the oral to 0.09 and the acetabulum to 0.063 mm. in diameter. The intestine has acquired the shape characteristic of the adult, the ovary has assumed definite form, the testes have become more prominent, and the cirrus sac is well defined at the anterior end although the posterior end is extended as a line of deeply staining cells reaching to and apparently connected with the ovary. No trace of uterus or vitellaria could be distinguished.

A comparison of *T. corti* with *T. aculeatus* and *T. solivagus*, the species which it most closely resembles, shows that the forms are about the same length; *T. corti* is narrower and thicker than the others. The oral sucker and pharynx are smaller in *T. corti* and the esophagus is shorter. Odhner (1902) says that in *T. solivagus* the descending and ascending limbs of the uterus cross to form a figure 8; in *T. corti* both crossed and parallel conditions are present. In *T. aculeatus* and *T. solivagus* the cirrus sac extends from the genital pore caudad to the ovary, in *T. corti* it extends only three fourths of the distance to the ovary. In *T. aculeatus* the lobes of the vitellaria are more separate and distinct than in *T. corti*, and in *T. solivagus* they are not definitely arranged. In *T. aculeatus* they do not extend as far anteriad or posteriad as in *T. corti*. The eggs of *T. corti* are about the same size as those of *T. solivagus* and smaller than those of *T. aculeatus*.

Some fifty individuals of this species, most of them immature, were found in the intestine of seven specimens of *Malacoclemmys lescurei* from Newton, Texas. An adult worm was obtained from the intestine of a single specimen of *Chrysemys elegans* from the same region. The species was also collected in June, 1910, at Havana, Ill., from the intestine of *Malacoclemmys geographicus*.

This species was named in honor of Dr. W. W. Cort.

Telorchis lobosus sp. nov. (Fig. 3)

Adults 1.67 to 2.6 mm. in length; 0.27 to 0.37 mm. in width, greatest width near center of body. Acetabulum circular, about two sevenths of total length from anterior end, in mounted specimens from 0.117 to 0.18 mm. in diameter. Pharynx 55 to 63 μ in diameter. Ovary oval, median, crosswise of the body, midway between anterior and posterior ends; from 40 to 70 μ in shorter and 74 to 85 μ in longer diameter. Small receptaculum seminis present, Laurer's canal passes directly dorsad, opening just above the ovary. Follicles of vitellaria massed together closely; lobes distinguished only at ends of areas. On left side vitellaria extend anteriad about one third of distance from ovary to genital pore, and posteriad about seven eighths of distance from ovary to cephalic testis. Vitellaria of right side do not extend so far anteriad or posteriad as those on left. Normal eggs measure from 18 by 32 μ to 19 by 36 μ . Testes oval, lobulated; their long axis perpendicular to that of worm; from 0.1 by 0.05 to 0.13 by 0.08 mm., very close together but not overlapping in any case. Caudal testis about its own length from posterior end of body. Cirrus sac extends from genital pore caudad to ovary; vas deferens not coiled as much as in *T. corti*.

In size and structure *T. lobosus* agrees most closely with *T. nematoides*, but a comparison with the description of Mühling shows specific differences. The forms are similar in the extent of vitellaria, cirrus sac, and size of eggs; but *T. lobosus* is smaller, shorter and flatter than *T. nematoides*, the suckers and pharynx are smaller, the esophagus is much shorter, and striking differences are noted in the ovary and testes.

Nine worms of this species were obtained from the intestines of two specimens of *Chelydra serpentina* from Walker, Iowa.

Telorchis medius sp. nov. (Figs. 2, 7)

Sexually mature worms 3 to 5.28 mm. in length; 0.35 to 0.48 mm. in width; body closely resembles *T. corti*. At anterior end cuticular spines 2.8μ in length, in rows 3μ apart. Acetabulum one fifth to one fourth of body length from anterior end; in mounted specimens longer than broad, measuring 0.1 by 0.11 mm. to 0.12 by 0.146 mm. Oral sucker circular or slightly oval, with either diameter greater, varying from 0.115 to 0.146 mm. Short prepharynx shows in well-extended specimens, in sagittal sections measuring 40μ in length. Pharynx broadly oval, 50 to 60μ long and 60 to 70μ broad, followed by long esophagus measuring 0.2 to 0.27 mm. Diverticula extend posteriad to point midway between caudal testis and posterior end of body, diameter 12 to 15μ . Ovary spherical or broadly oval, median, three sevenths of total length from anterior end; diameter 0.15 to 0.2 mm. Figure 7 shows relations of female genital apparatus in ovarian region. Vitellaria extend anteriorly to point midway between caudal end of cirrus sac and cephalic margin of ovary, and posteriad seven eighths of distance from ovary to cephalic testis; lobes usually distinguishable. Eggs 21 by 43μ ; near ovary slightly more spherical, measuring 26 by 42μ . Testes spherical or oval, 0.185 to 0.25 mm. in diameter. Cirrus sac extends from genital pore caudad four fifths to five sixths of distance to ovary. Vas deferens coiled more than in *T. lobosus* and less than in *T. corti*.

T. medius shows more morphological similarity to *T. corti* than to any other known species but the differences constitute sufficient ground for separation. *T. medius* is shorter, more uniform in width, has a much longer esophagus, the cirrus sac extends farther caudad, the acetabulum and ovary are nearer the middle of the body, and the vitellaria do not extend so far anteriorly, the eggs are larger, and considering the differences in the size of the worms the ovary and testes are also larger in *T. medius*.

Eleven mature and sixteen immature specimens were taken from the intestines of three dozen individuals of *Aromochelys odoratus* from Raleigh, N. C.

Telorchis diminutus sp. nov. (Fig. 8)

Mature worms 1.2 to 1.5 mm. in length; 0.2 to 0.25 mm. in width. Greatest width in acetabular region. Acetabulum circular or oval, two sevenths of total length from anterior end; 62 to 80μ in diameter. Oral sucker circular or oval, 75 to 90μ in diameter; pharynx from 30 to 40μ in diameter; esophagus 70 to 100μ in length. Ovary spherical, 60 to 80μ in diameter, caudal margin midway between anterior and posterior ends of body. Receptaculum seminis present, Laurer's canal about 30μ long, opens immediately caudad of ovary. Descending and ascending limbs of uterus usually interwoven and not in distinct fields. Vitellaria poorly developed, not arranged in lobes, extending from ovary posteriad two thirds to three fourths of distance to cephalic testis. Eggs measure 41 by 20μ . Testes spherical or slightly oval, usually longer in the anteroposterior axis, 58 to 76μ by 69 to 92μ . Cirrus sac extends posteriad from genital pore almost to region of ovary; very much coiled.

In general structure *T. diminutus* closely resembles *T. parvus* Braun, but the two species differ in many distinct items. They have approximately the same relative width and correspond closely in position of vitellaria and size of eggs; but *T. diminutus* is smaller, the suckers are larger, the esophagus is shorter, the ceca extend farther

caudad, the cirrus sac does not extend to the ovary as in *T. parvus*, and in *T. diminutus* the collecting duct of the excretory system is dorsal to the testes instead of passing between them in the shape of a letter S.

These parasites were obtained from the intestine of a single specimen of *Cinosternum pennsylvanicum* from Raleigh, N. C. Some thirty worms were found, about half of which were sexually immature.

Telorchis robustus Goldberger (Fig. 6)

The species was described by Goldberger in 1911 from a single specimen which was taken from the intestine of *Cistudo carolina* in Maryland. I have fifteen specimens collected in 1910 from the intestine of *Chrysemys elegans*. A study of these worms affords information which corrects and completes the description of Goldberger. Specimens 6 to 13 mm. in length; 0.8 to 1.3 mm. in width. Acetabulum 0.2 to 0.24 mm. in diameter, located about one fifth of total length from anterior end. Oral sucker usually slightly longer in antero-posterior diameter, 0.25 to 0.28 mm. in the larger specimens. Short prepharynx; pharynx 0.14 to 0.17 in diameter; esophagus short, apparent in well-extended specimens. Testes spherical or slightly oval, 0.4 to 0.5 mm. in diameter. Female reproductive organs as described by Goldberger except that Laurer's canal opens some distance posterior to the ovary and the vitellaria do not "extend in intercecal areas," but lie in extracecal margins of body. Eggs (not mentioned by Goldberger) average 15 by 29 μ .

Telorchis aculeatus von Linstow (Fig. 5)

The material consists of 13 specimens collected from the intestine of *Tropidonotus grahamii* in June, 1910.

A careful detailed comparison of the worms with the description and figure of *T. aculeatus* given by Braun (1901) leaves little doubt as to their specific identity and regardless of the wide difference in distribution and host I shall assign the specimens to that species.

SUMMARY

The study of abundant material, both adult and immature forms from the trematode genus *Telorchis*, including four species new to science and others from new hosts and localities, has given data for the first general discussion of the genus yet made. The sub-genera *Telorchis* and *Cercorchis* proposed by Lühe intergrade and can not be retained. *T. angustus* and *T. leptus* if correctly described should be removed to an independent genus. The new and some older species are discussed in detail.

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EXPLANATION OF PLATE

ABBREVIATIONS

<i>a</i> acetabulum	<i>p</i> prostate gland
<i>cs</i> cirrus sac	<i>ph</i> pharynx
<i>e</i> esophagus	<i>sg</i> shell gland
<i>cp</i> excretory pore	<i>sr</i> seminal receptacle
<i>g</i> genital pore	<i>sv</i> seminal vesicle
<i>i</i> intestine	<i>t</i> testis
<i>l</i> Laurer's canal	<i>u</i> uterus
<i>m</i> metraterm	<i>v</i> vitellaria
<i>o</i> ovary	<i>vr</i> vitelline receptacle
<i>od</i> oviduct	<i>vt</i> vitelline duct
<i>os</i> oral sucker	

All drawings are from camera lucida tracings except Figure 7, which is from a reconstruction.

Fig. 1.—Terminal section of the genital ducts in *T. corti*, showing cirrus sac and metraterm.

Fig. 2.—*T. medius*, ventral view, x 28.

Fig. 3.—*T. lobosus*, ventral view, x 36.

Fig. 4.—*T. corti*, ventral view, x 18.

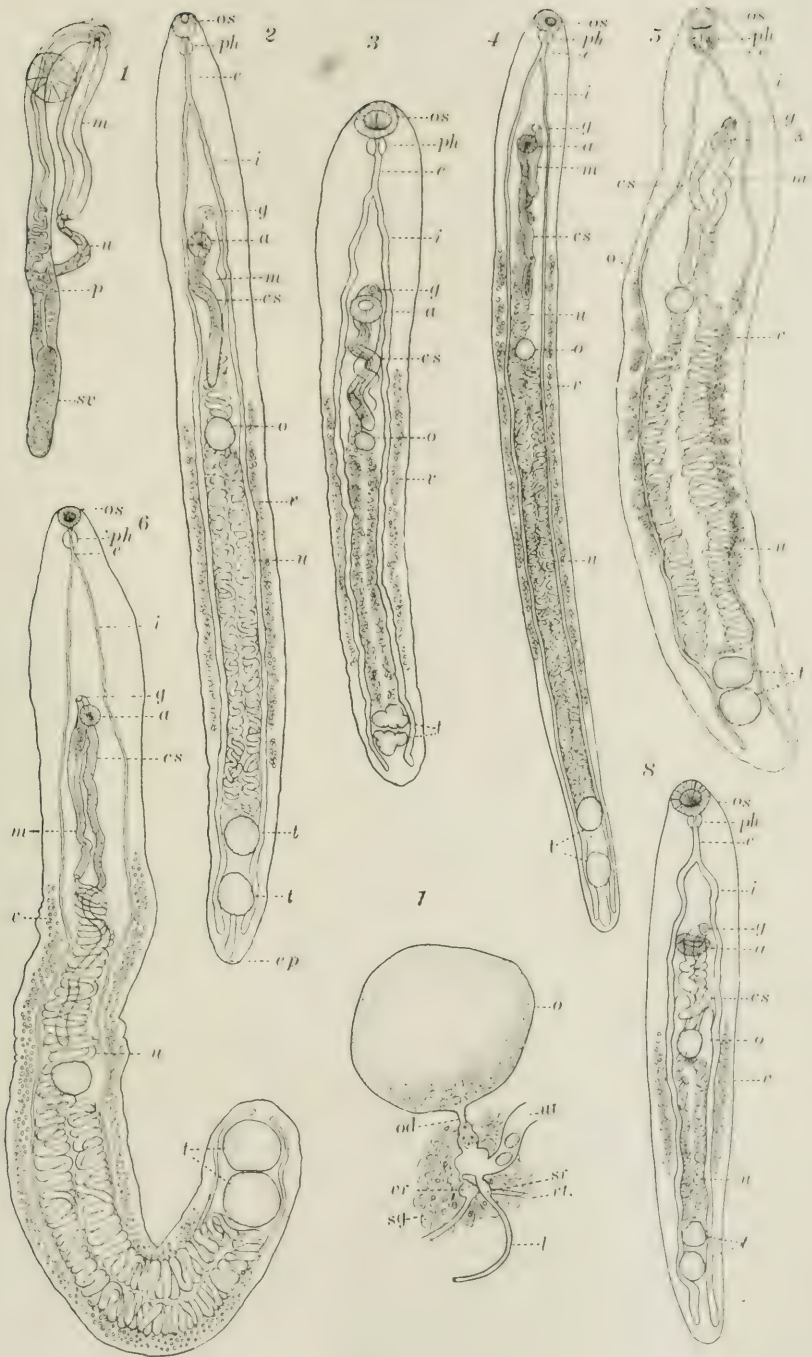
Fig. 5.—*T. aculeatus*, ventral view, x 18.

Fig. 6.—*T. robustus*, dorsal view, x 15.

Fig. 7.—Female genital apparatus, *T. medius*, from reconstruction of frontal sections, x 125.

Fig. 8.—*T. diminutus*, ventral view, x 56.

PLATE 1



Figs. 1-8

THE INSECT VECTOR OF UTA, A PERUVIAN DISEASE

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The disease commonly called *uta* in Peru is largely lupus vulgaris or tubercular lupus. It is this form apparently, or a complication with it, which results, when the infection becomes far advanced, in such disastrous cases as that of Perry Boyd, photographed on page 5, volume 5, of the *Inca Chronicle* (Cerro de Pasco, Peru; October, 1913) and reproduced in part on Plate 36, Figure 2, of the Harvard School of Tropical Medicine's report of 1913 expedition to South America.

The true *uta* without tubercular complication is evidently not a more serious affection than oriental sore, to which it is very closely allied. So far as we yet know it appears to be confined to the Andean region, occurring chiefly on the western slopes in Peru, though the name *uta* is also applied in certain districts of the eastern slopes to a similar affection. Probably the chief endemic focus of *uta* is the town of Otao, situated in the next canyon north of the Rimac valley and about opposite the point where Verrugas Canyon opens into the latter. Ugaz demonstrated the inoculability of *uta* in 1886.

Dr. Albert L. Barton, of Lima, Peru, evidently was the first to arrive at a correct diagnosis of *uta* as dermal leishmaniasis. Dr. Barton has shown to the writer his notes, made in 1910, recording the discovery of the specific organism in a case treated by him, then and there identified by him as *Leishmania*. These notes were not published, due both to lack of time from professional duties and to the belief that the organism was the same as that of oriental sore (*Leishmania tropica* Wright) already recorded and described.

Jan. 3, 1913, Dr. L. Velez Lopez announced in the local press of Lima that he had discovered in *uta* lesions what he called the "cuerpo leishman peruvianum." Jan. 13, 1913, Dr. E. Escomel announced that this was a new form, for which he proposed the name *Leishmania americana* (Lav. and Natt.-Larr., 1912) var. *uta* (*Crónica Médica*, Lima, 30: 414).

July 7, 1913, Gastiaturú and Rebagliati announced to the Academia Nacional de Medicina of Lima that they had found *Leishmania* in *uta* lesions (*Crónica Médica*, 30: 324). The organisms were shown to the writer at the time by the authors. These findings have been still

further verified by Strong et al (Report of Harvard 1913 expedition, p. 178).^{*} The nature of the affection has therefore now been abundantly demonstrated.

The question of distinctness of the leishmaniasis occurring on the eastern slopes of the Andes and in the low-lying forested country adjacent thereto is still open. Various names have been applied to this class of infection in different regions and districts. *Espundia*, *tiacc-araña*, *juccuya*, *quecño*, *llaga*, *apaicha*, *huaspi*, and *úlceras de los bosques* may be largely different names for the same infection. The term *espundia* is commonly applied in the montaña of Peru and Bolivia along the east slopes and base of the Andes, as well as farther north in the moist montaña. In the Pangoa montaña of Peru, the term *llaga* obtains. In the montaña of Paucartambo the affection is called *juccuya*; and in Apurimac, *quecño*. In the Urubamba valley of Cuzco department the term *tiacc-araña* obtains. In the lower tropical rain-forest region of eastern Peru similar infections go by the names of *apaicha*, *huaspi*, and *úlceras de los bosques*, according to locality. These last are described as superficial ulcers of the skin, which begin with itching roseate spots, the small acne-like tumors that result being painful. It should be noted that this description does not agree with that of oriental sore, the lesions of which are said not to be painful. Furthermore, the lesions of true *uta* are not painful.

The natives of Convencion province below Cuzco say that *tiacc-araña* is caused by the bite of a "minute spider" (*arañita*), whence the name. The "*arañita*" is probably a larval tick, less likely a *Trombidium*, but in either case is not necessarily the carrier of the infection. In other parts of Peru, the natives describe the carrier as a small hairy, whitish fly, which is called by them *uta* and *uta venenosa*.

Laveran and Nattan-Larrier demonstrated *Leishmania* in January, 1912, in smears from lesions of a case diagnosed as *espundia*, sent them by Dr. Escomel from Arequipa, Peru (*Bull. Soc. path. exot.*, 5:176), and proposed the name *Leishmania tropica* var. *americana* for the form. Wenyon confirmed these findings almost simultaneously in a case of *espundia* from Tambopata on the lower Rio Inambari of Peru (*Jour. London Sch. Trop. Med.*, I, No. 3). Dr. Carlos Monge M. has also given full particulars of his own findings of *Leishmania americana* in cases of *espundia* and *tiacc-araña* in 1912 and 1913 (Informe al Ministerio de Instrucción del Peru, 1912; *Crónica Médica*, Lima, April 30, Oct. 15, Nov. 30, 1913).

^{*} It is well to call attention to the statements with reference to *uta* on page 6 of this report: "Its etiology hitherto had not been determined. We were able to show that *uta* is due to a species of *Leishmania*." And on page 178: ". . . the parasite discovered by us as the etiological factor of *uta*." The authors have overlooked the earlier findings.—C. H. T. T.

It is not very probable that the true *uta* of the western face of the Andes is identical with the *espundia*, *tiacc-araña*, *juccuya*, *quecño* and *llaga* of the eastern slopes. It is still less likely that it is the same as the *bouba* (also wrongly spelled *buba*) or oral leishmaniasis of southern Brazil and northern Paraguay, known in the tropical forests of Brazil since 1759. The latter has been described by Splendore, who states, however, that it is undoubtedly to be identified with the *espundia* of Peru (*Bull. Soc. path. exot.*, 5: 436, 1912). Its specific organism has been named *Leishmania brasiliensis* by Vianna (*Mem. Inst. Oswaldo Cruz.*, 6: 41, 1914). The infection is stated to be contracted in the daytime in the forests of southern Brazil, and is believed to enter at any insect bite, or even at thorn scratches or other abrasion of the skin, though tabanids are indicated as the most probable and frequent agent of transmission. It is more likely that the Brazilian leishmaniasis is identical with the *apaicha*, *huaspi* and *úlceras de los bosques* of the low forest region of eastern Peru, rather than with the forms occurring higher up in the Andean valleys.

On the night of the discovery of *Phlebotomus verrucarum* Townsend, June 25, 1913, at San Bartolome, just below the mouth of Verrugas Canyon in the Rimac valley of Peru, I took some thirty specimens of *Forcipomyia utae* Knab on the inside of window panes of the railway station. These were placed in citrated artificial serum as captured. Twenty-seven specimens of the *Forcipomyia* were ground up finely in 2 c.c. of the citrated serum, warmed over flame, and injected into the ventral region of a guinea-pig, June 27, 1913, at 3:45 p. m., in the verruga laboratory at Chosica. Two injections of 1 c.c. each were made at points close together. This experiment was numbered 20. The pig was a male, born in the laboratory at Chosica, May 13, 1913, of parents from Jauja, Peru. The record of the experiment and material secured therefrom are as follows:

June 27.—Injection of 27 *Forcipomyia utae* as above detailed. Smear made from bodies of 4 gnats of same species from same lot, and numbered BS 22.

July 3.—Sore forming at point of injection.

July 5.—Sore about 1.5 cm. in diameter, subrounded, not raised, inflamed on edges, scabbed over.

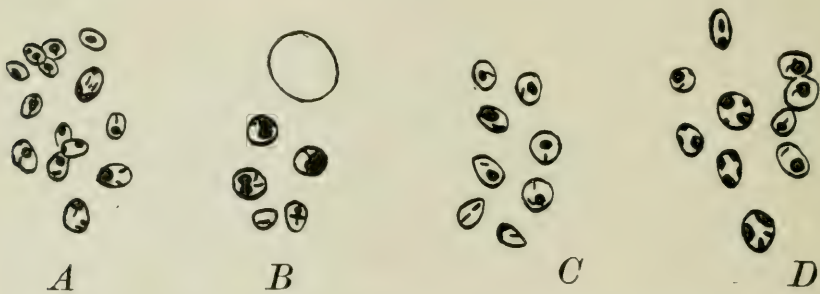
July 6.—Scab loosened easily, lifted at one side and smear made from exudation beneath, scab being let back in place. Scab dark colored, sore seems healing slowly. Smear numbered BS 23.

July 11.—Scab came away completely, and sore is seen to be covered over with a thin membranous epidermis.

July 15.—Sore healed over completely, but a small red pinhead papule has appeared at periphery of healed area on one side. This papule was well raised, 1.5 mm. in diameter, and reddish or pink. Left for further developments.

July 21.—Pig dead and discarded. Temperatures from June 3 to July 17 had been normal.

It was recognized throughout that this was not verruga, the smear from the sore seemed on examination to show nothing tangible, and the experiment was at the time deemed of no importance. The pressing requirements of the verruga investigation, especially the securing of wild flies of the *Phlebotomus* for injection in laboratory animals, prevented the proper study of this experiment. The two smears were not thoroughly examined till long afterward (November, 1913), when it was found that both showed a few bodies resembling *Leishmania*. The small pinhead papule at periphery of the healed area was then realized to have been quite certainly *uta*, and it probably contained numerous *Leishmania*. It was evidently of the same nature as the minute red papules described by Wenyon as appearing at the periphery of healed areas resulting from inoculations of oriental sore in man (PARASITOLOGY, 4: 279), and by others as following such inoculations in experimental animals.



Aug. 15, 1913, two females of *Forcipomyia townsendi* Knab came to light in my room in the railway hotel at Matucana, Peru, where I was engaged in securing nocturnal bloodsuckers for study in connection with the verruga investigation. They were placed at once in Gilson's fluid for fixation, later imbedded and sectioned Nov. 6, and 9, 1913, the four slides being numbered Sn. 20 to 23 inclusive. These showed *Leishmania* that could hardly be doubted, and caused the restudy of the two smears already mentioned.

Even with these findings I have allowed the results of this experiment to rest thus far unannounced, due to doubt of the organism being that of *uta*. There seemed always the possibility that the organism was merely a stage of a *Herpetomonas* confined to the gut of the gnats. Furthermore, *Forcipomyia townsendi*, the species which was sectioned, occurs at Chosica, which point was supposed to be outside the *uta* zone, though this may be doubted. At the time, however, I regarded *uta* as never occurring at Chosica, which threw further doubt on the findings.

Recently I have gone anew over both the material and my original notes of 1913. As a result I believe that I have substantial proof in the slides (smears and sections) of the transmission of *Leishmania uta* Escomel by both species of the *Forcipomyia*. The data are as follows:

Smear 22 (bodies of 4 *Forcipomyia utae* from San Bartolome).—Contains several forms of the *Leishmania*, as shown in Figure A. A minute flagellate form is mentioned in my original notes as found in this smear.

Smear 23 (exudation from sore of pig 20, injected 9 days previously with 27 *Forcipomyia utae* from San Bartolome of same lot as preceding).—Forms of *Leishmania* with trophonucleus dividing, shown at Figure B. Very scarce.

Section 20 (longitudinal sections of whole body of 1 female *Forcipomyia townsendi* from Matucana).—Shows numerous *Leishmania* in the abdominal region, apparently only in the gut; none to be seen in thoracic region or head. Oval to slightly pointed at one end, as shown in Figure C. Several of a large pointed flagellate form are mentioned in my original notes as found attached to wall of rectum.

Section 21 (same as preceding) did not result well.

Section 22 (transverse sections of abdomen of the other female *Forcipomyia townsendi* from Matucana).—Shows quite numerous *Leishmania* in various stages in gut, some of which are dividing, shown in Figure D.

Section 23 (debris from sections of 22).—Shows developmental stages of *Leishmania*.

The *Leishmania* is evidently voided by the gnats from the anus while feeding, and infection must take place when the bites are rubbed. Apparently there is no other possible method in which this organism can be transmitted by biting insects, since it exists in no form that can reach the salivary glands or proboscis.

The geographical range of *uta* on the west slopes of the Andes coincides quite well with that of the two species of *Forcipomyia*, which were found to occur from Chosica to Matucana, and may extend higher than the latter point. The districts in this range come mainly under the head of temperate sierra valleys, and include the deep humid quebradas of the Andes noted since ancient times as the seat of the disease. The seasonal prevalence of *uta*, given as November to April or the rainy months, coincides with the period of greatest prevalence of the *Forcipomyia*.

Two cases were noted by me and clinically diagnosed as *uta* infection, the first of which shows a history quite certainly traceable to the bites of the *Forcipomyia*, the second being in all probability due to the same cause. It was not possible to take smears from these cases at the time that I saw them, hence they lack microscopical findings.

Messrs. Chadwick and Holstein, of the Peruvian Central Railway, lay over night in their private car on the tracks at San Bartolome about March 20, 1913, and have testified that many very small gnats, both the *Phlebotomus* and others, entered their car on this occasion and

bit the inmates. Mr. Gutierrez, of Lima, a minor official of the road and an intelligent and educated man, was one of the inmates. He states that he was bitten on the hands; that he saw some of the gnats, as at least a part of the bites was received early in the evening before he retired; that the gnats that bit him while he was awake were of a dark color, as seen by the lamplight, wings not white, smaller in size than the *Phlebotomus*, but rather stouter than same and with shorter legs. Following this experience, there appeared at the bites spreading sores. I examined these sores March 29, 1913, and can testify that they bore every appearance of tropical ulcer. They were eight or ten in number, irregularly rounded, 0.5 to 1.5 cm. in diameter, inflamed at edges, not raised, faintly scabbed. I prescribed citrine ointment. The sores lasted fifteen days or so, then healed. When I saw the case again on July 1, 1913, three months after, the sites of the sores showed as purplish scars.

The second case was that of Dr. ———, assistant physician at the Cerro de Pasco Hospital, a native of Canada, who spent some days and nights in the railway hotel at Matucana, about March, 1914. He remembered being bitten at night, did not see the insect, supposed it to be a mosquito, but acknowledged that he had seen no culicids. As a matter of fact, they were absent. A half dozen or more sores appeared on his wrists and forearm, not so large as in the case of Mr. Gutierrez, and not of such a virulent appearance. They were about 0.5 to 0.8 cm. in diameter, well raised, well scabbed, the scabs being dark, not spreading and not inflamed, but they persisted for many weeks, longer than in the preceding case, finally disappearing.

Both of these cases I regard as mild infections of true *uta*, without tubercular or other complication. Such cases demand the same treatment as oriental sore, and are no more serious; perhaps not as serious as that affection. Citrine ointment, applied promptly to all bites and skin abrasions in the *uta* zones, will probably effectually prevent any advanced development of such infection. Dr. J. Leonidas Samanez states that the treatment of the developed lesions by applications of albuminate of mercury is superior to all others (*Crónica Médica*, 18: 89-90, 1901). More recently neosalvarsan has been claimed by Almenara as a specific against the organism in the general circulation, and necessary to prevent its spread from advanced lesions to new localities (*Crónica Médica*, 30: 476-7, Nov. 30, 1913).

The incubation period of *uta* appears to be shorter than that of oriental sore. Note that only eighteen days elapsed between injection and appearance of the pinhead papule in above Experiment 20. The disease, when uncomplicated with other infection, may also run its course in much less time.

That other insects than the *Forcipomyia* transmit *Leishmania* in Peru is very probable. Certain tabanids probably carry the organism of *apaicha* in the eastern rain-forest region. At Chachapoyas, Amazonas province, Peru, the natives accuse *Ornithodoros* n. sp. aff. *turicata* Duges (det. Nuttall and Warburton) of producing *uta* by its bites. This tick, like *O. talaje* in Mexico, inhabits the mud walls of the dwellings and sallies forth at night to bite the inmates after the manner of the bedbug; it is probably not concerned in this transmission. *Simulium* appears never to be implicated; likewise *Stomoxys* seems excluded. Both are too generally distributed. Fleas have been shown to be possible carriers of *Leishmania*; but fleas, bedbugs and ticks seem excluded in the case of *uta* and other Peruvian leishmaniasis, since the lesions are practically confined to the exposed parts of the body—hands, face and feet. Culicids are contraindicated, since all experiments tend to show that *Leishmania* degenerates in their gut. At all events, it appears certain that *Forcipomyia utae* and *F. townsendi* are implicated as vectors of true *uta* on the western side of the Andes.

In conclusion, attention should be called to one important point. As late as 1915, investigators of leishmaniasis have questioned whether the specific organism is not really a stage in the development of a *Crithidia* or *Herpetomonas* normally confined to the gut of insects, normally conveyed from insect to insect, and only accidentally transferred to man. In the present case the fact that most species of *Forcipomyia* are normally insect-biters, attacking caterpillars and certain other insects, would tend to confirm this view. *Forcipomyia utae* and *townsendi* are very abundant at times during the humid season. It may easily transpire that these gnats, while normally confining their attacks to other insects, have become accustomed, during their periods of greatest abundance, to transfer their attacks to man, due to a shortage of food-supply in the insect fauna requisite for the needs of their increased numbers.

SUMMARY

(1). The disease known as *uta*, occurring on the west face of the Andes in Peru, has been proved to be due to a *Leishmania*.

(2). Two species of *Forcipomyia*, native to the western Andean region, appear to be proved capable of transmitting the *Leishmania* of *uta*.

(3). It is highly probable that the various forms of leishmaniasis thus far known are due to as many species of herpetomonads originally parasitic in the gut of the insect-carriers concerned, and that, with regard to the occurrence in man, these herpetomonads are as yet in the stages of parasitism ranging from habitually abnormal or frequent to merely accidental or infrequent.

FILARIA CINGULA PARASITIC IN THE SKIN OF
CRYPTOBRANCHUS ALLEGHENIENSIS *

FREDERIC H. KRECKER

So far as I am aware the only worms reported to be parasitic in the skin of the members of the genus *Cryptobranchus* are an encysted *Bothriocephalus* larva found by Leuckart and a Nematode, *Filaria cingula*, mentioned by von Linstow. Both parasites infested *Cryptobranchus maximus*.

The description of *Filaria cingula* given by von Linstow is taken from a single specimen and is rather incomplete, nevertheless his specimen and a worm found by me in *Cryptobranchus allegheniensis* appear to have certain points in common. The most important difference is that *Filaria cingula* has a vulva whereas I have not yet been able to discover one in the worms from *Cryptobranchus allegheniensis*. My specimens are not as perfect as they might be so it is possible that further search will disclose the presence of a vulva. In the meantime, for the sake of conservatism, it may be best to consider the specimens as being specifically identical with *Filaria cingula*. It is worthy of note that the *Cryptobranchus maximus* in which von Linstow found *Filaria cingula* came from Japan whereas the host of the worms here under discussion is found in the Ohio river. In view of von Linstow's meager description some account of these parasites may be of value.

Six specimens of *Cryptobranchus* were taken from the Ohio river at Marietta, Ohio, in March, 1914, and kept in an aquarium. Within the next two months worms were found on four of the animals. They were threadlike, 15 to 25 cm. long, and whitish in color. The oldest individuals are little more than cuticular tubes filled with living young. These older worms protruded for varying distances from the skin of the host. Younger individuals did not protrude. The embedded portions of the worms were barely covered by the epidermis of the host. They caused a loosely sinuous, light colored elevation of the skin, the course and extent of which differed in each case. The worms were located at various places on the dorsal, lateral and ventro-lateral surfaces of the trunk, on the tail, and even on the head.

The more detailed description about to be given is based in large part upon the best specimen at hand which I shall hereafter refer to as the complete specimen. This worm is 20 cm. long. It has a practically uniform diameter of 0.53 mm. The anterior end is bluntly rounded

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(Fig. 1). The mouth is terminal. There is one dorsal and one ventral lip. These project anteriorly for 0.028 mm. and are roughly triangular, being 0.04 mm. wide at the base and 0.02 mm. wide at the tip. In the two oldest specimens I noticed two blunt hooks inserted in each lip. They appear as two parallel, refracting lines and may be followed to the base of the lip. In one of the two worms they protrude a very short distance from the tip of the lip. In the other individual they are retracted. In this condition it is hard to distinguish them because their refractive index is about the same as that of the lips. Inability to detect them in the other specimens is probably due to this cause. At the base of the lips the body is 2 mm. wide. It increases in width very rapidly up to a point 1 cm. towards the posterior where it is 0.53 mm. wide, which is the practically uniform diameter of the remainder of the worm. Near its posterior extremity the body tapers down rather suddenly to a diameter of 0.2 mm. which it retains for 0.3 mm. and then ends in a rounded point 0.075 mm. wide. The anus is terminal (Fig. 2).

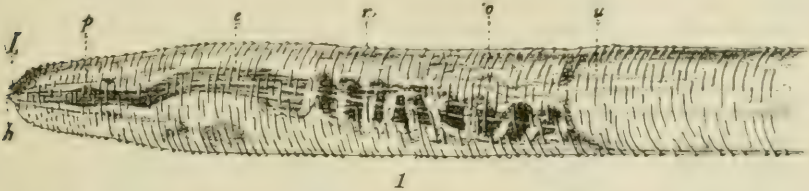


Fig. 1.—Optical section of the two anterior millimeters; *l*, lips; *p*, pharyngeal bulb; *o*, ovary; *e*, esophagus; *u*, uterus; *h*, hooks; *r*, ridges.

The cuticula is characterized by an embossment in the form of low, rounded, transverse ridges with bluntly rounded ends. These are confined to the dorsal and ventral surfaces, each area being 0.42 mm. wide and extending the length of the body. In a surface view of the worm the ridges are most evident along the sides of the body where they appear as lateral thickenings that project out 0.01875 mm. Elsewhere they are hard to discern unless the light strikes them at the proper angle. They vary in length, some of the shorter ones being 0.059 to 0.09 mm. long, and the longer ones 0.18 to 0.28 mm. The width varies slightly with the individual, those on the complete specimen having an average width of 0.033 mm. They are arranged end to end in rows with spaces varying from 0.0187 to 0.056 mm. between them. These intervals are usually overlapped by a ridge in an adjacent row. The space between rows varies from 0.037 to 0.075 mm. The embossment begins at the base of the lips in the form of rounded papillae-like elevations which become successively longer and more definitely aligned until at a point about 1 mm. from the base of the lips the previously described arrangement is attained. The ridges gradually become less

distinct on the narrow region at the posterior end of the body and the latter half of this region is smooth (Fig. 2). There is a smooth space on each side of the body 0.4 mm. wide which separates the dorsal and the ventral embossed areas throughout their extent (Fig. 4). At both ends of the body all these areas become proportionately narrower. Scattered over the surface of the lateral fields are a number of extremely minute, rounded papillae. Along both the dorsal and the ventral edges of each field 0.093 mm. distant from the respective embossed areas, there are some slightly larger papillae which are more or less regularly arranged in two rows. The papillae of one row alternate with those of the other. The distance between those of a row averages approximately 0.056 mm. The rows are about 0.037 mm. apart.

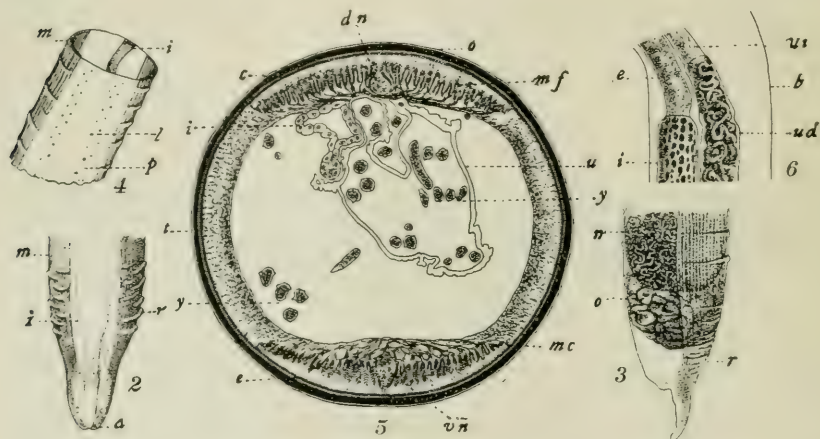


Fig. 2.—Optical section of posterior end; *a*, anus; *i*, intestine; *m*, muscles.

Fig. 3.—Posterior end of complete specimen; *o*, degenerate ovary; *r*, indistinct cuticular ridges.

Fig. 4.—Surface view of lateral field; *l*, lateral field; *p*, cuticular papillae in rows.

Fig. 5.—Cross section through uterus; *dn*, dorsal nerve; *vn*, ventral nerve; *mc*, muscle cell; *mf*, muscle fibril; *i*, intestine; *o*, outer layer of cuticle; *c*, inner layer; *u*, uterus; *y*, section of young; *e*, epidermis; *t*, tissue of lateral field.

Fig. 6.—Junction of esophagus with intestine; *c*, esophagus; *i*, intestine; *ud*, partially distended uterus; *ui*, immature uterus; *b*, body wall.

There are two layers in the cuticle, an inner layer about 4μ thick and an outer layer which is probably one fifth as thick (Fig. 5). In surface view of the lateral field two types of very fine striations are visible. One of them follows the circumference of the body and occurs at very short intervals. The other striations are, if anything, finer than the preceding and run diagonally. Separation of the two layers shows that the outer layer is homogeneous and that striations are in

the inner layer. This layer stains very deeply in Delafield's hematoxylin whereas the outer layer does not take the stain but has a slightly amber hue. Beneath the cuticula is a single layer of extremely narrow columnar epidermis cells about 0.018 mm. in height.

The alimentary tract is the nearly straight, approximately centrally located tube characteristic of the nematodes. In the complete specimen it is displaced by the greatly distended uterus and winds in long open spirals. There is a narrow pharyngeal tube approximately 0.375 mm. long in which at a point 0.26 mm. from the mouth there is a spindle-shaped bulbular enlargement. This is 0.15 mm. long and midway it has a maximum width of 0.11 mm. Anterior to the bulb the width of the pharynx is 0.055 mm. Posterior to the bulb the width is 0.11 mm. but it almost immediately widens out into the esophagus which may be said to begin 0.375 mm. from the mouth. The esophagus is 0.13 mm. wide and extends posteriorly for 15 mm. to the intestine. In cross section it is triangular. There is a sharp constriction of the esophagus at its junction with the intestine (Fig. 6). The intestine has an average diameter of 0.187 mm. It ends in a short rectum. In the complete specimen the lumen of the intestine is obliterated in both the intestine and the adjoining portion of the esophagus, but these structures lie free in the coelom. Further back the intestine is represented by a brown, flat band which adheres to the body wall. In the younger individuals the intestine is free and retains its tubular shape throughout.

The reproductive system consists of a uterus and two ovaries. As previously mentioned the presence of a vagina or a vulva is still in doubt. The uterus when fully distended with young entirely fills the coelom with the exception of 2.7 mm. at the anterior end and 0.56 mm. at the posterior end of the body. Its wall is approximately 0.0042 mm. thick. In the less mature individuals the uterus is only partially distended. This is particularly well shown in a specimen in which the distention begins 15 mm. from the anterior end of the body. The distended portion is only 0.2 mm. in diameter and anterior to this it decreases to 0.09 mm. within a distance of 0.2 mm. The young in the enlarged region are the size of those in the complete specimen but in the zone between the narrow and the distended regions there are extremely small young.

The young average 0.33 mm. in length and 0.014 mm. in width. Their anterior end is blunt; the posterior one-fourth of the body tapers rapidly into a sharp hair-like point. I saw none enclosed in a capsule although many of them were coiled. In most parts of the uterus they maintain a constant wriggling, but in places they are so tightly packed as to leave an impression on the body wall. Individuals liberated in tap water retained their activity.

At each end of the uterus there is a single, slender, tubular ovary. The anterior ovary of the complete specimen begins 1.12 mm. from the anterior end of the body and runs posteriorly in a series of coils about the esophagus to the uterus. It then extends forward slightly beyond its point of origin and then again turns back and joins the anterior end of the uterus which is 1.59 mm. from the anterior end of the body. The diameter of the ovary at its tip is 0.056 mm.; for the greater part of its extent it is 0.09 mm.; and at its junction with the uterus 0.11 mm. The posterior ovary in the complete specimen has degenerated and is represented by an irregular mass of tissue.

The muscles are restricted to two broad bands, one dorsal and the other ventral, each corresponding in extent to the dorsal and the ventral embossed areas of the cuticula. In the mid-dorsal and the mid-ventral lines each band is interrupted by a nerve cord. In a surface view of the animal the muscles give the effect of longitudinal corrugations (Fig. 3). In cross sections of the body 36 muscle cells are distinguishable in each band, there being 18 on each side of the median line.

In the material at command no excretory tubes can be distinguished, but I hesitate to deny their presence entirely. The lateral field, where such tubes are usually found, is occupied by an apparently homogeneous mass of connective tissues of a rather loose texture. This field has a width of 0.14 mm., or practically one-fourth of the circumference of the body.

The nervous system was also only partly distinguishable. There are two longitudinal nerve cords, one in the mid-dorsal and the other in the mid-ventral line (Fig. 5). I could not determine the nature of the anterior termination of the cords in any of the worms because of the poor histological condition of the sections in this region.

There are some stray notes regarding the life history which may be of interest. As stated before, the worms are viviparous and the young can survive in water. They are apparently liberated by the disintegration of the parent's body. In two of the worms that portion which protruded from the host had been attacked by a fungus and was so far disintegrated that the body barely remained intact. Young worms which had broken out of the uterus were crowded into the coelom of this region. It is an interesting fact that among the two lots of *Cryptobranchus* which I have had under observation there have been no specimens which showed signs of infection when they were received. Both lots were taken from the same place at the same time of the year, although one lot was taken a year later than the other. In both cases signs of the parasites were observed about a month after their hosts had been caught. At this time the worms caused an almost imperceptible thread-like elevation of the skin. A month later in each case the parasites were clearly visible and had apparently about reached

the end of their growth. Both lots had been kept in filtered water although the supply in each case was from a different source. The young parasites probably entered the skin of their hosts during the summer from the water in which the latter lived.

The percentage of the individuals infested must be very great since of the two lots which have come under my observation, there being six specimens in one and four in the other, all but two of the individuals harbored one or more of the parasites. Apparently the presence of the parasites is a matter of no concern to the host. There are no evidences of injury or of disturbance in the tissues other than the narrow tube formed in the epidermis of the host. The inner lining of this tube glistens and is smooth except for slight indentations made by the cuticular ridges on the parasites.

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THE LIFE HISTORY OF *GONGYLONEMA SCUTATUM*

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In April, 1911, the writers undertook some investigations in regard to insects as intermediate hosts of parasites. The investigations of the senior author were carried on at the Experiment Station of the Bureau of Animal Industry at Bethesda, Md., and in the laboratories of the Bureau at Washington, and later at Colorado Springs, Colo. Those of the junior author were carried on at Colorado Springs, Colo. Particular attention was paid to the dung beetles, as it seemed evident that these insects in working through fresh feces as is their habit, would have the first opportunity to ingest eggs of worms parasitic in the digestive tract of cattle, sheep, and other live stock. Such beetles as species of *Aphodius*, furthermore, appeared small enough to be readily ingested by grazing animals, and the beetles' habit of flight from one manure deposit to another offered chances for such ingestion, as the beetles' flight commonly terminates on grass and herbage with which they might readily be swallowed by cattle or sheep.

The dissections by the senior author of *Aphodius femoralis*, *A. granarius*, *A. fimetarius* and *Onthophagus hecate* resulted in the finding of encysted larval nematodes in the body cavity. These cysts were about 0.5 mm. in diameter and as many as 8 were found in one *Aphodius* and 15 in one *Onthophagus hecate*. One larva which was measured was 2 mm. in length and 50μ in thickness. Viewed from in front the head shows a narrow mouth aperture elongated dorso-ventrally and surrounded by a chitinous border, which is oblong quadrangular in outline with rounded corners, and measures about 12μ dorso-ventrally and about 8μ from side to side. A short distance posterior of the edge of the chitinous border are 2 sub-dorsal and 2 sub-ventral papillae. The chitinous border of the mouth is raised above the surrounding surface of the head and resembles a projecting flange when the head is viewed from the side. The slender pharynx is 40μ long. The esophagus measures about 1.5 mm. in length, and is differentiated into a slender anterior portion about 225μ long, and a more granular posterior portion of somewhat larger diameter. The anus is about 100μ from the tip of the tail. The latter is blunt and is supplied with two or three very small short conical processes. The excretory

pore is about 200μ from the anterior end of the body. About 140μ from the anterior end of the body the esophagus is surrounded by a nerve ring.

In June a number of larvae of *Aphodius* spp. were examined, and in these were discovered some young nematodes which agreed perfectly with the unhatched embryos of *Gongylonema scutatum*. The embryo of *Gongylonema scutatum* is very distinctly annulated in the head region on the side opposite the mouth. The mouth is not terminal, but is a triangular aperture on one side of the head, with a curved hook-like process projecting from it. The tip of the tail is bluntly pointed.

The finding of these newly hatched larvae in the same host beetles as the encysted larvae was fairly good evidence that they were of the same species and, in view of the failure to find them in adult beetles, seemed to indicate that the eggs of the worm were principally ingested by the beetle while it was in the larval stage.

In July both of the writers were in Colorado and the examination of *Aphodius coloradensis*, *A. vittatus*, and *A. granarius* showed the presence of an encysted larval nematode exactly similar to that found at Bethesda. These beetles were collected from sheep manure at points 17 miles east and 75 miles northeast of Colorado Springs.

The observations in regard to the finding of *Gongylonema* larvae in larval and adult dung beetles were briefly alluded to in the Twenty-eighth Annual Report of the Bureau of Animal Industry for the year 1911, as follows:

"Important facts have been determined bearing upon the life history of the gullet worm of sheep and cattle."

In 1912 the encysted larval forms of *Gongylonema* were again observed in the dissection of insects in connection with other investigations in Colorado, but no further work was carried on that year.

In 1911 an experimental feeding was made by the senior author. Four or five larval *Gongylonema* were fed to a white mouse on May 13 and 14 more were fed to this mouse on May 17. On June 14, 32 and 28 days, respectively, after these feedings, the mouse was killed and examined. No worms were found. This result tended to show that the parasite is not transmissible to mice and that the larvae were not those of *Spiroptera obtusa* whose larvae, occurring in the meal worm, show very striking similarities to those which the present writers assumed belonged to *Gongylonema*.

In 1913 an experimental feeding was made by the junior author at Colorado Springs. During the two months from May 10 to July 9 inclusive, a sheep was fed a total of over 250 specimens of *Alphodius coloradensis*, *A. congregatus*, *A. fimetarius*, *A. granarius*, *A. inquinatus*, and *A. vittatus*. A dissection of a large proportion of these beetles showed a very small number of them to contain encysted *Gongylonema* larvae.

Three of the larvae obtained on dissection were also fed to this sheep. This sheep was killed November 18 and was found to have 7 *Gongylonema scutatum* in the esophagus, together with the characteristic lesions showing where a few others had been at one time. A companion sheep kept under identical conditions for a year and a half, but not fed any specimens of *Aphodius* did not develop any infection with *Gongylonema*. Thirty-five lambs raised under experiment conditions very much the same as those of the two sheep mentioned above were killed during the course of the three years 1911 to 1913 inclusive, and these were all free from *Gongylonema*. This furnishes an abundance of checks in support of the experimental finding that species of *Aphodius* act as intermediate hosts of *Gongylonema scutatum*.

During the summer of 1914 some additional work was done in the investigation of this life history. Cattle weasands heavily infested with *Gongylonema scutatum* were sent in from Indianapolis, Ind., by Dr. G. W. Butler, the egg-bearing worms cut into small fragments, mixed with small quantities of bread or other food and fed to specimens of *Aphodius* and to croton bugs, *Ectobia germanica*. The day after the insects were exposed to infection in this way, empty shells of *Gongylonema* eggs were found in the intestine, and the following day numbers of free embryos were found. Other eggs were found unhatched in the intestine and in the feces of the insects, but these were obviously eggs which had not yet developed to the infective stage. These findings demonstrated that the eggs would hatch when ingested by the adult as well as by the larval *Aphodius*.

The young larvae found two days after exposure to infection were about 250μ long and were apparently increasing in size. In the course of a week the larvae found were very much thicker. At the end of two weeks the larvae were about three times as thick as the original embryos and were apparently on the verge of an ecdysis. At this time they show a complete alimentary tract. The esophagus is about three-eighths to four-ninths of the entire body length, and is surrounded by a nerve ring a short distance in front of its middle. The rectum is a well-marked structure of rather large diameter, and posteriorly is closed by a plug of tissue which projects from the ventral surface of the body. This plug marks the location of the anus and is one-sixth of the body length from the posterior end. About this time there is an ecdysis and the cephalic annulation is lost.

At the end of three weeks the head is more pointed but the flange-like margin of the lips is not yet developed. The rectum is no longer prominent, but the button marking the position of the anus persists. The larvae are now much longer.

At the end of about a month the larvae are encysted in the final stage. The head has the structure described in the first part of the paper and the anal button is lost. In favorable specimens cervical papillae may be seen about half way between the nerve ring and the anterior end of the body.

An experimental feeding to croton bugs of eggs of *Gongylonema* from the gullet of a hog, gave substantially the same results. The larvae were encysted in the final stage at the end of a month.

A rabbit was fed with three *Gongylonema* larvae on one occasion and with two on another. Two months after the first feeding and one month after the second, the rabbit was killed and the mouth, pharynx, and stomach were examined. No worms were found. A guinea-pig was fed with three *Gongylonema* larvae on one occasion and three more on another. Five weeks after the first feeding and three weeks and two days after the second feeding it died. No worms were found.

August 18 a sheep was fed eleven *Gongylonema* larvae from a croton bug and a hog was fed a croton bug containing possibly fifty larval *Gongylonema*, the larvae having been developed by feeding eggs of *Gongylonema* collected from cattle. On August 25 the same sheep and hog were fed more croton bug material heavily infested with similar larvae. The hog was killed October 17, but showed no infection. The failure to infect the hog with the nematode from sheep and cattle is suggestive of a specific infectivity and strengthens the idea that the hog nematode is a distinct species. The sheep was killed November 23 and the gullet found heavily infested with *Gongylonema*, the females of which were mature and full of eggs.

While the work noted above was in progress, a very interesting paper appeared, dealing with the life history of another species of *Gongylonema*. Fibiger (1913) published a note in which he stated that he had found in rats a gastric carcinoma etiologically related to a species of *Spiroptera*. A year later, Fibiger and Ditlevsen (1914) published their complete study of the worm itself and the lesions attributed to it. The worm in question, called by them *Spiroptera* (*Gongylonema*) *neoplastica*, should be called *Gongylonema neoplasticum*. *Gongylonema* is a well established genus and there is no reason to question the propriety of including this species in *Gongylonema*, notwithstanding its lack of one characteristic of this genus, namely, the presence of cervical papillae. It is even not impossible that cervical papillae may be present, as these structures are frequently very difficult to distinguish in some species of nematodes and may be overlooked in numerous specimens, finally being discovered when a specimen happens to be turned into just the right position.

Fibiger and Ditlevsen have made an excellent study of the life history of this worm. It was found in the first instance in rats, but it appears to be communicable to rodents generally as it was transmitted to the following: *Mus decumanus*, *Mus rattus*, *Mus musculus*, *Lepus cuniculus*, *Cavia cobaya*. The parasite occurred in the squamous-celled epithelium of the anterior portion of the digestive tract, including the mouth, tongue, esophagus and fundus of the stomach. In these regions the worm gave rise to a proliferation of the epithelial elements, originating as a circumscribed or diffuse hypertrophy associated with a slight inflammation, going on to the formation of papilloma, and terminating in distinct carcinoma with occasional metastases.

The eggs produced by the female worm are passed in the feces of the infested rodent and were first found to be ingested by *Periplaneta americana*, but were also found infective for *Periplaneta orientalis*, *Ectobia germanica* and *Tenebrio molitor*. Twenty days after the ingestion of the eggs by the insects, the fully developed larvae are found coiled in the muscles of the prothorax and limbs. It will be noted that this site is different from that of *Gongylonema scutatum* larvae. The location of the embryonic and larval forms after the first day following the ingestion of the eggs and up to the time they are found in the musculature of the prothorax and limbs was not determined.

It is evident from the above that the life history of the two species, *Gongylonema scutatum* and *Gongylonema neoplasticum* is much the same in that the larval stage is spent in insects, at least one of which, *Ectobia germanica*, is common to both, and that the adult worm is found in the epithelium of the gullet in the primary host in both cases. The worms differ in that the larval stage of the rodent nematode is found in the musculature of the insect host, while the larval stage of the ruminant nematode is found encysted in the body cavity. They also differ in that the rodent nematode commonly occurs in the tongue, mouth and cardiac portion of the stomach as well as in the esophagus. Finally, the rodent nematode has the unusual power of producing neoplastic changes in its primary host, while there is yet no evidence that the ruminant nematode is more than a rather innocuous parasite.

The life history of *Gongylonema scutatum* and *G. neoplasticum* is strikingly similar to that of *Spiroptera obtusa*, which occurs in its adult stage in the intestine of rats, mice and similar rodents.

Leuckart (1867: 113-115) and Marchi (1871) found that the larval development of this parasite occurs in the meal worm (larva of *Tenebrio molitor*). The eggs, which resemble those of *Gongylonema* and contain similar embryos, when swallowed by meal worms hatch out and release the embryos. These embryos pass through the wall of the

alimentary tract, and develop in the midst of the fat surrounding it, becoming enclosed in connective tissue cysts. The larval development is complete in about six weeks after ingestion of the eggs. The fully developed larva measures from two-thirds of a millimeter to nearly a millimeter in length. The head, as described, is supplied with two triangular papillae curved on their inner surfaces, and surrounding the mouth except laterally. The tip of the tail is supplied with several small conical papillae. The excretory pore is about 100μ and the base of the esophagus about 300μ from the anterior end of the body.

Judging from Marchi's description and figures one of the most striking differences between the full-grown larvae of *Spiroptera obtusa* and *Gongylonema* is that the esophagus of the former is only about one-third the length of the body, whereas the esophagus of the latter is fully two-thirds the body length.

As a postscript it may be noted that since this paper was read at a meeting of the Helminthological Society of Washington, Dec. 17, 1914, an additional intermediate host of *G. scutatum* has been found, namely, *Onthophagus pennsylvanicus*. Beetles of this species collected from sheep pastures near Vienna, Va., during the summer of 1915 were found to be commonly infested with the encysted larvae.

SUMMARY

The eggs of *Gongylonema scutatum* present in the feces of sheep and cattle infested with the adult parasite, hatch out when swallowed by insects of various species.

The larvae thus released from the eggs, pass into the body cavity and reach the final larval stage in about a month. In this stage the larva is coiled into a spiral and is enclosed in a capsule about half a millimeter in diameter. The length of the fully developed larva is about 2 mm. and the esophagus equals about two-thirds the body length. The mouth, elongated dorso-ventrally, is surrounded by a flange-like chitinous border.

Sheep fed upon insects containing these larvae became infested with *Gongylonema*. A hog fed upon croton bugs artificially infested by feeding with eggs of *Gongylonema* from cattle failed to become infested. A mouse, rabbit and guinea-pig fed with *Gongylonema* larvae from beetles found in sheep manure, or from croton bugs artificially infested by feeding *Gongylonema* eggs from cattle, also failed to become infested. Failure to produce infestation in these various animals indicates that the *Gongylonema* of sheep and cattle (*G. scutatum*) is not transmissible to hogs, mice, rabbits or guinea-pigs.

Gongylonema larvae have been found in various species of dung beetles collected from sheep manure, namely, *Aphodius femoralis*, *A. granarius*, *A. fumentarius*, *A. coloradensis*, *A. vittatus*, *Onthophagus*

hecate, and *O. pennsylvanicus*. They have been developed in various species of *Aphodius* and in croton bugs (*Ectobia germanica*) by feeding the eggs of *Gongylonema scutatum* from cattle. The feeding of eggs of *Gongylonema* from the gullet of a hog (presumably *G. pulchrum*) to croton bugs also resulted in the development to encysted larvae.

Under natural conditions the usual intermediate hosts of *Gongylonema scutatum* are probably dung beetles of various species.

The life history of *G. scutatum* is similar to that of *G. neoplasticum* of rats, mice and other rodents, the intermediate stage of the latter having been found by Fibiger and Ditlevsen to develop in roaches (*Periplaneta americana*, *P. orientalis*, and *Ectobia germanica*) and in a beetle (*Tenebrio molitor*). It is also similar to that of another rat and mouse parasite, *Spiroptera obtusa*, whose intermediate host was found by Leuckart and Marchi to be the larva of a beetle (*Tenebrio molitor*).

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NOTE ON THE STAGE OF *PIROPLASMA BIGEMINUM*
WHICH OCCURS IN THE CATTLE TICK,
MARGAROPUS ANNULATUS

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The organism herein described, which is believed to be a stage in the life cycle of *Piroplasma bigeminum*, was found in engorged female cattle ticks (*Margaropus annulatus*) removed from cattle in September, 1913. These ticks, which were being used as controls in a series of experiments at the Bureau of Animal Industry Experiment Station, Bethesda, Md., on the action of arsenical dips, had been immersed in water, dried, and maintained in the laboratory in petri dishes. Under such circumstances cattle ticks ordinarily behave in the normal manner; that is, by far the greater number live until oviposition is fully completed. In the present instance, however, certain of the lots showed an unusual mortality, as a result of which microscopical preparations were made and the organism which is the subject of the present paper discovered.

This, as seen in the figure, is a cigar-shaped body, with one end pointed and the other differentiated into a sort of cap. The ratio between the length and breadth varied considerably, but it is probable that this variation is due at least in part to the exigencies of fixation.

The cap, which is placed at what is probably the anterior end, varies a good deal in appearance in the different specimens. In some cases it has the shape of a crown with a minute, pointed process arising from its median portion (*A*). In others it consists merely of a narrow shell fitting over the broad end of the parasite (*D*), while again it consists of a rounded body resting upon the balance of the cell like the proto-merite of a polycystid gregarine (*B*, *C*). As a matter of fact, these parasites are strikingly like minute gregarines, and may appropriately be termed gregarinoids. In some cases (*A*, *D*) the substance of the cap was much denser and more homogeneous than that of the balance of the cell, whereas in other cases (*B*, *C*) this distinction was not evident.

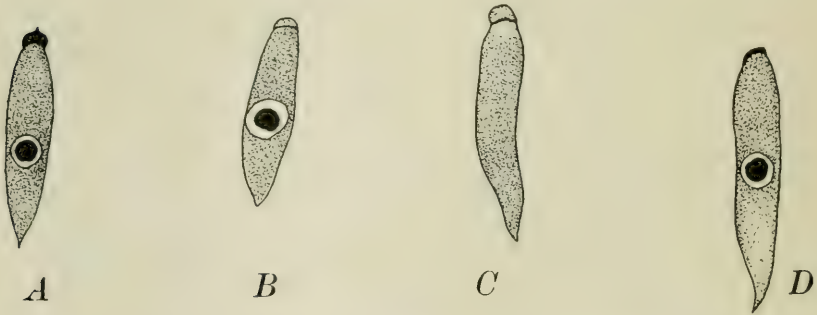
The cytoplasm is finely alveolar, thus following the protozoan type. In many of the specimens there were large vacuities, but these are probably to be accredited to the technique. An ectoplasmic layer is probably present, but it was not possible to demonstrate it satisfactorily.

The nucleus occupies a median position. It is of the vesicular type and inclosed within a thick and very distinct membrane. Within, a large rounded karyosome is always present.

Fifty specimens, selected at random, were measured. The extremes in length were 7μ and 15μ ; the mean length 11.2μ . The distribution of these fifty specimens, with reference to their length, was as follows:

Length in microns	No. of Specimens
7	1
8	1
9	6
10	10
11	10
12	13
13	4
14	4
15	1

Christophers (1907) has described the life cycle of *Piroplasma canis* in the dog tick (*Rhipicephalus sanguineus*). In this, one of the most conspicuous phases is what Christophers calls the club-shaped body, which he describes on pages 56 to 61, and illustrates by a number of figures on his plate 2. The precise resemblance between these, and the



forms herein described from *Margaropus annulatus*, leave no doubt as to their being corresponding stages in the life histories of *Piroplasma canis* and *Piroplasma bigeminum*.

According to Christophers, the club-shaped bodies are derived directly from the parasites ingested by the tick in blood from the dog. The difference in both size and morphology between the intracellular form of *Piroplasma canis* and the club-shaped bodies is considerable, but Christophers figures several intermediate stages, and there seems to be no reason to question his conclusions. The club-shaped bodies were found in the gut of the dog tick, and, more abundantly, in the ovaries, oviducts, and eggs. Christophers describes two forms, as follows:

"(a) Rather rigid thorn-like bodies resembling on a very small scale certain gregarine trophozoites. This resemblance is largely due to the presence of a peculiar disc-like structure armed with cusps carried at the anterior extremity. They are sometimes motionless, but usually exhibit rather slow movements, especially a side to side flap-like action of the tail portion. The protoplasm is transparent, slightly refractile,

and free from large granulations, and in it a clear area (the nucleus) can often be made out.

"The disc has the appearance of a boring organ. It carries four or five cusps, one of which is situated centrally, whilst the others are arranged around the periphery. Immediately behind this structure the parasite is often constricted so that a kind of neck is formed.

"(b) Leech-like forms more club-shaped than those just described and executing very active movements. They possess a swollen end which is often attached to the slide or cover glass and a thin end which is kept in constant motion like the head of a leech. They also undergo modified ameboid movements, the shapes depicted in plate 2, figure 2, being characteristic.

"These bodies are most numerous as a rule about the oviducts, and a few can be found here when not to be detected elsewhere. They also occur in the ovary and may be seen lying with ova not yet free in the lumen, and in more mature ova. By careful search they can also be found in fresh preparations of the gut.

"In stained preparations a greater variety of forms is apparent, but the two forms mentioned, those with and those without a disc, are distinguishable. Since intermediate stages are not difficult to find it is probable that both are identical in nature, the disc bearing forms being the more mature stage."

Finally, according to Christophers, these club-shaped bodies enlarge, become irregularly rounded, and transform themselves into what he designates as the zygote. This term, however, is singularly unfortunate, since no sexual process is described. The "zygotes" by a series of steps which Christophers describes in detail, break up into sporozoites, these later stages of the life cycle taking place in the salivary glands of the tick.

Koch (1906), studying *P. bigeminum*, figured and described what are clearly the same bodies, although he says nothing about the curious cap or crown present at one end of the parasite. Koch found these elements both in engorged female ticks three days after removal from the cow, and in the eggs. According to his account, they are the later rather than the earlier stages in the evolution of *Piroplasma* in the cattle tick, but he did not follow them further than the stage here under consideration.

Kleine (1906) and Nuttall and Graham-Smith (1908) cultured *Piroplasma canis*, and obtained developmental forms much like the earlier stages of *P. bigeminum* in the tick, as described by Koch. The cigar-shaped body, however, has so far only been seen in either ticks or tick eggs.

These various observations are not wholly accordant, nor do they seem to cover the entire process. Minchin (1912: 384), however, has endeavored to put them together and to formulate an outline of the life history of *Piroplasma* in the tick, and to this the reader is referred.

My own observations on *Piroplasma bigeminum* in *Margaropus annulatus* concern only the club-shaped bodies or gregarinoids. As already stated, these differ somewhat amongst themselves with regard to the morphology of the cap at the anterior end, but, as Christophers suggests, such differences are probably merely a matter of the stage of development. In my material from *Margaropus annulatus*, the condition of the cap shown in *D*, was quite infrequent, from which it may be surmised that these gregarinoids were in an earlier rather than a later stage of their evolution. Furthermore, as already stated, the gregarinoid was the only stage that I was able to find, or, at least, to recognize.

In addition to their occurrence in smears made from the ticks themselves, the gregarinoids were also found in preparations made from the crushed eggs. In the eggs, however, they were very scarce and could be picked up only with the greatest difficulty. In crushed seed ticks, hatched from eggs laid by ticks of the infected lots, I have not been able to find either the gregarinoids or any element which can be identified as belonging to *Piroplasma bigeminum*.

It has been noted that the ticks in which the parasites were found had shown an unusually high mortality, it being on this account that they were examined. This suggests that the *Piroplasma* is pathogenic for the tick as well as for the cow.

In consequence of the discovery of the parasite of Texas fever in the tick, a series of experiments was carried out and, although they were wholly negative, the failure is of itself of some significance, and a brief résumé may not be out of place.

The ticks which showed the high mortality were collected early in September from a certain cow (No. 1040 of the Bureau of Animal Industry series). The microscopical preparations were made on September 22 from ticks collected on September 4, or 18 days after reaching maturity. The weather being then warm all of the ticks which had survived were either in the midst of oviposition or had nearly or quite completed it. It was obviously beside the point to search for the parasite in the bodies of ticks which had laid all their eggs since such ticks merely shrivel and die. Moreover, the material was not abundant.

In consequence, the eggs of the batches of ticks (collected September 2, 3, 4 and 5) which showed the high mortality, and which were moreover known to be infected, were kept in appropriate containers and permitted to hatch. On Oct. 18, 1913, microscopical preparations

were made of a number of the seed ticks, and the balance of them were placed on cow 1040. These ticks began to come to maturity on November 24, and collections were made daily from November 24 to November 28. Microscopical preparations were made daily of these ticks from one to seventeen days after their removal from the cow, but the parasite could not be found. In consequence, the experiment was abandoned.

These negative results are in line with the results of observations made in 1908, during the course of which a number of infectious ticks were examined without detecting anything which could be identified as belonging to the life cycle of *Piroplasma bigeminum*. The inference seems to be that of a given number of so-called infectious cattle ticks, only a certain proportion actually harbor the parasite. It is easy to understand how this might take place. A female cattle tick lays several thousand eggs and in order that her entire brood should be infected it would be necessary that each egg receive at least one parasite. Such a condition could fail of realization in two ways. In the first place, the engorged female might not harbor as many parasites as eggs, and in the second, the distribution of the parasites would need to be remarkably accurate to insure that each and every egg became infected.

It is also evident, from the experiments herein outlined, that whereas a given lot of ticks may be heavily infected, the offspring of these may be negative at least so far as a microscopical examination is concerned.

It may finally be noted that a spirochaete has several times been detected in cattle ticks and that it was present in the lot which showed the heavy infection with *Piroplasma*. *Spirochaeta theileri*, first discovered by Theiler (1904) in cattle in Africa, is stated to be the causal agent of a mild disease. It is known to be transmissible by *Margaropus decoloratus*, and further that adult infected ticks of this species can transmit the spirochaete to their offspring. *Margaropus decoloratus* and *Margaropus annulatus*, the former in Africa and the latter in the United States, play similar rôles in the transmission of cattle diseases. Hence it may be suggested that the spirochaete occurring in *Margaropus annulatus* is *Spirochaeta theileri*.

SUMMARY

A parasitic protozoan was found in smears made from female cattle ticks (*Margaropus annulatus*), and from crushed eggs which they had deposited. The parasite has the form of a minute polycystid gregarine, and is believed to represent the stage of *Piroplasma bigeminum* occurring in the tick. It is essentially like the form figured and described by Koch as present in engorged female ticks and their eggs, and also like the form of *Piroplasma canis* found by Christophers in

Rhipicephalus sanguineus. In the present case, it is of interest to note that the female ticks in which the parasites were found showed an unusual mortality, suggesting that the parasite is pathogenic for the tick as well as for the cow. In addition to the gregarinoid parasite a spirochaete was found in the ticks. This parasite not heretofore reported from the United States is perhaps the same as the form known as *Spirochaeta theileri*.

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SOCIETY PROCEEDINGS

THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON

The twenty-seventh regular meeting of the Society was held at the residence of Doctor Hall, Oct. 22, 1915, Doctor Hall acting as host and Dr. N. A. Cobb as chairman.

The following resolution was approved by the Society:

WHEREAS, Unnecessary changes in the names of host animals add to existing difficulties and confusion in the study of the parasites of these hosts, therefore be it

Resolved, That the Helminthological Society of Washington approves and endorses the official lists of such generic names as authorized by the Zoological Congress at Gratz, and urges that helminthologists use the approved names in their publications.

Dr. B. H. Ransom presented the following note on a third American case of *Dipylidium caninum* in man:

Under date of Sept. 15, 1915, Dr. L. T. Cassidy of Norwich, Conn., forwarded to the Zoological Division of the U. S. Bureau of Animal Industry for determination some segments of a tapeworm passed by a Polish child aged 1½ years. On examination the tapeworm proved to be *Dipylidium caninum*. This apparently is the third case in man to be reported from the United States. Stiles (1903c) reported a case in a 16-months old child at Detroit, Mich., and Riley (1910)* a case in an 11-year old boy at Ithaca, N. Y.

This tapeworm is a common parasite of dogs and cats, but comparatively rare in man, altogether less than 100 human cases having been reported. Most of the cases of infestation in man have been of children less than 3 years old. Infection undoubtedly occurs as a result of ingesting infested lice (*Trichodectes canis*) or fleas (*Ctenocephalus canis*). The chances of swallowing these insects are of course greater in the case of children than of adults, which probably explains the greater frequency of infestation in the former. As an indication of the frequency of infestation in dog fleas it may be of interest to note that in July, 1912, I examined 21 fleas from a dog which was heavily infested with *Dipylidium caninum*; two of the fleas were found to harbor cysticercoids, one in one case, and seven in the other.

Doctor Ransom also presented the following list of parasites from the Island of Guam:

During the last year the Zoological Division of the U. S. Bureau of Animal Industry has received from Dr. L. B. Barber of the Guam Agricultural Experiment Station, a number of specimens of parasites for identification. The following species were represented among these specimens:

Trematoda.—*Fischoederius cobboldii* (?), cow; tentative determination. *Fasciola hepatica*, cow. *Fasciola* sp., carabao; resembles *F. gigantica* in form, but is smaller.

Cestoda.—*Davainea echinobothrida*, chicken.

Nematoda.—*Oxyuris equi*, horse. *Stephanurus dentatus*, pig. *Metastrongylus apri*, pig. *Trichuris* sp., pig; apparently not *T. crenatus*. *Tetrameres fissispinus*, chicken. *Cheilosporura nasuta*, chicken. *Ascaridia perspicillum*, chicken. *Heterakis vesicularis*, chicken. *Oxyspirura mansoni*, chicken.

* Science, 31: 349.

Arthropoda.—*Menopon trigonocephalum*, chicken. *Goniocotes gigas*, chicken. *Dermanyssus gallinae*, chicken. *Haematopinus tuberculatus*, carabao. *Margaropus caudatus* (?), cow; tentative determination.

Protozoa.—*Theilaria parva* (?), cow; tentative determination.

Dr. C. W. Stiles presented a note by Stiles and Graves on the lung capacity of children in the city of X. It was found on spirometer examination of 1,618 white school children, that from 6 to 13 years old (primary and grammar school age) the boys average from 100 to 200 c.c. greater lung capacity than the girls. From 14 to 17 years (high school age—an athletic period) the boys have progressively from about 300 to about 1,100 c.c. greater lung capacity than the girls, a striking increase.

From 6 to 13 years old inclusive, the yearly increase in the lung capacity of the girls is very similar to that of the boys, but at 14 there develops a distinct decrease of this increase, and from 14 to 17 years inclusive, the annual increase averages distinctly less than for the years 6 to 13.

The decrease of the increase at 14 years in the girls follows immediately on the average age of beginning menstruation (13.2 years) and corresponds with the decrease of the increase in height (sitting and standing) and weight.

There is a slight irregularity of the increase curve at 11 in both boys and girls, corresponding to the irregularity found for the same year in the curves for height (sitting and standing) and weight in the boys, and for sitting height in the girls.

In the case of both the boys and the girls, children from homes provided with better sanitation (sewer) have a tendency (total, 15 to 9; boys 8 to 4, girls 7 to 5; estimated in year groups) to greater lung capacity than the children from homes with poorer sanitation (privy; total, 9 to 15; boys 4 to 8, girls 5 to 7).

In cases of intestinal infection it was not evident that hookworm, *Ascaris lamblia*, or *Endameba coli* has any noticeable effect on the spirometer tests. While pupils with whipworm infections showed a preponderance of tests lower than the average, the number of cases is so small that conclusions are of doubtful value.

Doctor Stiles stated that findings formerly reported to the effect that chewing tobacco decreases and smoking increases with improved sanitation has been found to be true for city children as well as country children.

He presented another note setting forth the fact that in a study of the weight and the standing and sitting heights of a number of children, those with light hookworm infestations were found to be below the average in 91 markings and above in 68.

Doctor Stiles presented the following findings in an extensive study of the blood of 295 boys, from 6 to 17¼ years old, and of 279 girls in the city of X: The red blood cells are below the accepted normal for adults, being 4,617,000 for the boys and 4,678,000 for the girls. In 234 boys from homes with good sanitation (sewer), the red count was 4,633,000; in 51 boys from homes with poor sanitation (privy), the red count was 4,591,000. In 200 girls from homes with good sanitation (sewer), the red count was 4,752,000; in 74 girls from homes with poor sanitation (privy), the red count was 4,498,000. The lowest red count for a boy was 2,912,000; the lowest for a girl was 3,536,000. The hemoglobin averaged 86 per cent. for the boys and 87.4 per cent. for the girls. In 234 boys from homes with sewer the hemoglobin was 86.7 per cent.; in 51 boys from homes with privy the hemoglobin was 84.4 per cent. In 200 girls from homes with sewer the hemoglobin was 87.7 per cent.; in 74 girls from homes with privy the hemoglobin was 87.5 per cent. The highest hemoglobin index among the boys was 115 per cent. and the lowest 52 per cent.; the highest hemoglobin index among the girls was 128 per cent. and the lowest 40 per cent.

Although the standard number of white cells is regarded as 5,000 to 7,000, the average for the 295 boys was 8,012, and that for the 274 girls was 7,734. In a general way the increase in white cells is indicative of infection, poor condition, and the like. The 234 boys from homes with sewer showed a white count of 7,680; the 51 boys from homes with privy showed a white count of 8,687. The 200 girls from homes with sewer showed a white count of 7,731; the 74 girls from homes with privy showed a white count of 7,771. A differential white count, the most extensive ever made on children, was also part of the study. The average count for the 295 boys was as follows: Polymorphonuclear neutrophils, 52.06; eosinophils, 6.32; large mononuclears, 6.41; small mononuclears, 32.53; transitionals, 2.18; basophils, 0.47. The average for the 279 girls was as follows: Polymorphonuclear neutrophils, 53.2; eosinophils, 5.43; large mononuclears, 5.92; small mononuclears, 32.89; transitionals, 2.11; basophils, 0.51. The extreme high counts for the different types of cells in individual children were as follows: Polymorphonuclear neutrophils among the boys, 79; among the girls, 78.8; eosinophils among the boys, 27.2; among the girls, 26; large mononuclears among the boys, 32.5; among the girls, 36.5; small mononuclears among the boys, 65.8; among the girls, 60; transitionals among the boys, 10.4; among the girls, 7.6; basophils among the boys, 2.4; among the girls, 7.5. The extreme low counts for individual children were as follows: eosinophils, none; large mononuclears, none; transitionals, none; basophils, none; neutrophils, 18; small mononuclears, 2.

Details of this blood study will be published in *Public Health Reports*.

Doctor Hall presented notes on the following subjects: The city of X in the county of Z; A case of spurious parasitism.

Doctor Cobb exhibited a Spencer portable microscope.

Doctor Cobb presented a note bearing on parthenogenesis. Maupas in 1900 published a study of such nematode species as usually present only females, and in which the same gonad produces sperm cells and later eggs. Cobb calls this kind of hermaphroditism, syngonism, stating that, including the cases observed by Schneider, Bütschli, Claus, Maupas, himself, and others, the number of syngonic nematode species has now risen to several scores, and that there are excellent reasons for believing that syngonism is very common among free-living nematodes—is in fact the prevailing condition in numerous large genera. He suggests that supposedly parthenogenetic forms in general should be carefully reexamined with a view to determining whether some of them are not actually syngonic. He exhibited a figure of one of a series of syngonic free-living nematodes, showing consecutive processes of sperm and egg production, and said that he had series of species in which the spermatozoa to be found are smaller and smaller until they reach the optical limits of present instruments and methods, so that he finds himself in the position of being unable to assert the non-existence of spermatozoa in certain nematodes simply because he does not succeed in finding them. The researches naturally suggest the possibility that small spermatozoa may in the past in some cases have been overlooked.

Doctor Cobb also presented a drawing showing the mechanism of the spinneret of a free-living nematode. This mechanism, not heretofore understood, consists of a vesicle with an outlet controlled by a "needle-valve"—not, however, a free part as in machine valves. A somewhat needle-like plug seems to be forced into the outlet aperture by the pressure of the caudal secretions passing into the vesicle. To open the valve and permit a flow of secretions, the "needle" is withdrawn by retractor muscles passing from the proximal end of the needle obliquely forward to the dorsal wall of the tail.

MAURICE C. HALL, *Secretary*.

NOTES

The Annual Report for 1914 of the United Fruit Company Medical Department furnishes interesting data for the parasitologist. The seven divisions of the medical service cover fairly well the territory bordering on the Caribbean Sea. Conditions represented in the report concern the properties and employees of the company and are probably distinctly better than those in adjacent territory not so controlled. The data covering diseases caused by animal parasites have been collated from the complete tables of the report and are presented below in tabular form. Some records are more elaborate or more carefully worked out than others; and the absence of any entry under a given heading does not demonstrate the non-occurrence of the parasite or the disease at that place. None the less the report gives an interesting and valuable survey of the prevalence of animal parasites in man within this area of which relatively little is known in this respect. The figures are obtained from a grand total of 127,809 patients of whom 15,406 were treated in hos-

	Hospitals — Panama Division	Costa Rica Division		Guatemala Division		Colombia Division		Honduras Division		Hospitals and Dispensaries, Cuba (a)	Hospitals and Dispensaries, Cuba (b)	Steamship Service, Northern Division	Steamship Service, Southern Division
		Hospitals	Dispensaries	Hospitals	Dispensaries	Hospitals	Dispensaries	Hospitals	Dispensaries				
Malaria*.....	857	1,049	2,200	673	5,244	558	1,558	141	6,902	799	104	119	217
Amebic dysentery.....	8	1	5	73	1	3
Myasis, nasal.....	...	2	3	2	...	8	4
Ancylostoma.....	100	238	9	405	11	48	86	66	...	1	2	...
Ascaris.....	3	15	9	119	2	1	60	8	43	3	...
Strongyloides.....	1	1
Tapeworm.....	...	1	1	2	2	2
Other intest. parasites	1	28	2	2
Schistosoma.....	...	1
Seabies.....	2	10	67	2	47	...	60	2	58	5	2
Chiggers.....	4	70
Myasis, skin.....	...	1	1	20
Ground itch.....	1	4	23	1	3	...	47
Elephantiasis.....	...	2	1	1
Dhobie itch.....	4	20	359	...	109	...	8	...	131	2	7
Tropical ulcer.....	30	19	54	1	9	476	1	1
Venomous bites and stings.....	...	1	32	1	42	4	109	...	12	24	12
Snake bites.....	1	5	13	2	1	2	4

* Excluding cases classed as clinical malaria at hospitals.

pitals, 91,324 in dispensaries and 21,079 on steamships. The report is a valuable document and the company is to be congratulated on the organization and efficiency of its medical service. Similar data are not available in the large majority of our own highly educated and civilized states.

CONSOLIDATED LABORATORY REPORTS

	Pan- ama Divi- sion	Costa Rica Divi- sion	Guate- mala Divi- sion	Colom- bia Divi- sion	Hon- duras	Cuba (a)	Cuba (b)
Blood examinations.....	2,374	7,643	2,240	780	660	892	..
Estivo-autumnal.....	532	1,117	338	192	270	205	46
Tertian.....	441	467	321	193	22	335	..
Quartan.....	173	6	106
Mixed.....	18	6	9	2
Stool examinations.....	1,971	6,212	2,996	423	774	36	..
Ancylostoma.....	124	1,172	879	(80%)	(70%)
Ancylostoma and Trichocephalus.....	182	72	3	..
Ancylostoma and Ascaris.....	36	25
Ancylostoma, Ascaris, and Tricho- cephalus.....	53	31
Ancylostoma and Strongyloides.....	26	1	..
Ancylostoma duodenale.....	132	76	95	7	..
Ascaris lumbricoides.....	81	403	525	39	17
Trichocephalus dispar.....	226	1,048	358	22	73	8	..
Ascaris and Trichocephalus.....	34	2	36	0	..
Ascaris and Oxyuris.....	9
Strongyloides intestinalis.....	67	123	76	91	21	7	..
Oxyuris vermicularis.....	2	41
Schistosoma.....	1	1
Tapeworms.....	3*	1	1
Ameba.....	7	314	34	156
Ciliated monads.....	5	372	51	122†	3
Balantidium coli.....	1	19	7
	‡	§					

Also Ancylostoma, Ascaris, and Strongyloides, 1; Ancylostoma, Trichocephalus, and Strongyloides, 20; Ancylostoma, Ascaris, Strongyloides, and Trichocephalus, 4; Strongyloides and Ascaris, 4; Strongyloides, Ascaris, and Trichocephalus, 3; Strongyloides and Trichocephalus, 19.

* One each Taenia saginata, Hymenolepis nana, Dibothriocephalus latus.

† With Ascaris, 1; with Ancylostoma, 121.

‡ Cercomonas was recorded in 9 out of 2,353 urinalyses.

§ Amebae were recorded in 1 out of 6,148 urinalyses.

¶ Apparently no differential diagnosis between the two human hookworms.

RATE OF GROWTH OF THE BEEF TAPEWORM
IN HUMAN BEINGS

WHILE lecturer in zoology at Potchefstroom Agricultural School, South Africa, the author had an opportunity of determining the rate at which a tapeworm will grow in human beings and, as there seems to be very few references in literature, it was thought a short comment might be of interest.

One of the students at the school was treated for tapeworm and passed most of the worm, which was 9 feet 6 inches in length. The head, however, was not passed, and from the width of the smallest segments passed, there could not have been more than 6 inches of the worm left in his body. The date was carefully noted, but the student was not treated until such time as he was again suffering from the tapeworm. When treated the second time, the entire worm was passed. The worm then measured 19 feet 9 inches in length, showing a growth of about 19 feet 3 inches in seventy-two days. On obtaining the head, it was determined to be the beef tapeworm, *Taenia saginata*.

WILLIAM MOORE, University of Minnesota.

PSOROPTIC OTACARIASIS—PSOROPTES CUNICULI

Full grown gray and white rabbit, female. Attention was drawn to the animal because of drooping ears, being the only animal among forty so affected. The animal was caught and examined, when it was found to be affected by some parasitic disease. The ears were enormously thickened and completely filled with dry, dirty gray crusts. At the base of the ears were areas free of hair and covered with thin small scabs, which on removal gave a raw, bleeding surface. The scabs in the ears were difficult to remove, and when finally removed in toto caused a good deal of pain and small punctate hemorrhages. The scab consisted of dry, horny, crescentic, speckled white and gray layers, each layer containing numerous eggs and minute parasites. By microscopic examination, the parasite proved to be a mite and which later, on closer examination, seemed to be *Psoroptes cuniculi* (Psoroptic otacariasis).

The animal was thoroughly cleansed, received daily washes of hot bichlorid of mercury 1:200, dried, and a thick layer of carbolic vaselin rubbed over the ears. Complete recovery with no infection of the other animals.

Boston, Mass.

JEROME A. HONEIJ.

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LA FAMILLE DES THELAZIIDAE

A. RAILLIET, ALFORT, PARIS

Dans l'importante superfamille des Spiruroidea, on peut dès à présent établir un certain nombre de coupes correspondant à des familles.

C'est ainsi qu'ont été constituées déjà les familles des Spiruridae et des Acuariidae. De même le genre *Tetrameres* Creplin peut être considéré comme le type d'une famille des Tetrameridae; le genre *Hedruris* Nitzsch, celui d'une famille des Hedruridae; les genres *Ancyracanthus* Dies. et *Ancyracanthopsis* Dies. méritent d'être groupés en une famille des Ancyracanthidae, etc.

Il me paraît utile enfin de constituer une famille des Thelaziidae pour les genres du type *Thelazia* Bosc. A côté de ce type viennent en effet se ranger naturellement les genres *Ceratospira* Schneider et *Cystidicola* Fischer, ainsi que deux autres genres nouveaux, *Schistorophus* et *Serticeps*. Et l'on peut même en rapprocher provisoirement le genre *Oxyspirura* Drasche, ainsi que deux autres groupes à créer: *Galeiceps* et *Rhabdochona*.

Famille des THELAZIIDAE.—Spiruroidea. Tête nue ou pourvue, soit d'expansions cuticulaires, soit d'un revêtement en forme de casque. Bouche tantôt sans lèvres, tantôt à six petites lèvres, parfois à deux seulement, suivie en général d'un vestibule allongé ou d'une courte capsule buccale. Œsophage composé, dans la règle, de deux parties distinctes.

Mâles à queue généralement obtuse, avec ou sans ailes latérales (bourse), portant de chaque côté une rangée linéaire de nombreuses papilles préanales parfois couplées; papilles postanales peu nombreuses; 2 spicules presque toujours très inégaux.

Femelles à queue généralement mousse; deux utérus; vulve à situation très variable. Ovipares ou vivipares.

Habitat.—Région orbitaire des Mammifères et des Oiseaux; tube digestif ou vessie aérienne des Oiseaux ou des Poissons.

Genre type: *Thelazia* Bosc.

Le tableaux ci-après peut servir de clé pour la détermination des genres :

1—Spicules égaux	<i>Galeiceps</i>
Spicules inégaux	2
2—Bouche pourvue de lèvres ou dents.....	3
Bouche sans lèvres	5
3—Bouche à six petites lèvres papillifères.....	<i>Serticeps</i>
Bouche à deux lèvres ou dents.....	4
4—Tête ornée de lobes cuticulaires.....	<i>Schistorophus</i>
Tête nue	<i>Rhabdochona</i>
5—Mâles à ailes caudales (bourse).....	6
Mâles sans bourse; courte capsule buccale.....	7
6—Vestibule allongé; papilles préanales couplées.....	<i>Cystidicola</i>
Courte capsule buccale; papilles préanales simples.....	<i>Ceratospira</i>
7—Queue obtuse, arrondie; vulve antérieure.....	<i>Thelazia</i>
Queue pointue, oxyuriforme; vulve postérieure.....	<i>Oxyuris</i>

Genre *Thelazia* Bosc, 1819 (*Thelazius* Bosc, 1819; *Thalazia* de Blainville, 1819).—Bouche sans lèvres, suivie d'une *capsule buccale*; bord antérieur de la capsule retroussé en dehors et découpé en six festons par des échancrures dont quatre paraissent occupées par un petit organe papilliforme très réfringent. Deux papilles céphaliques latérales et quatre submédianes.

Mâle à queue obtuse ordinairement recourbée en crochet, *sans ailes latérales*; un grand nombre de *papilles préanales* dont une médiane, impaire, au-dessus du cloaque; trois ou quatre (?) papilles postanales. Deux spicules inégaux.

Femelle à queue conique mousse, arrondie, portant deux papilles latérales à son extrémité. *Vulve située antérieurement*, un peu en arrière de la terminaison de l'oesophage; deux branches utérines dirigées en arrière. Embryons éclosant dans les utérus.

Habitat.—L'habitat normal est représenté par les canaux exréteurs des glandes lacrymales des mammifères, d'où les Vers s'échappent assez souvent pour glisser sous les paupières ou à la surface de l'oeil; on en a signalé exceptionnellement à l'intérieur du globe oculaire. Certaines formes semblent se rencontrer sous la membrane nictitante des Oiseaux.

Espèce type: *Thelazius Rhodesii* Desmarest, 1827.

A.—Espèces des Mammifères.

1.—*Thelazia rhodesi* (Desmarest, 1827).—Syn.: *Thélazie* de Rhodes Bosc, 1819; *Thelazius Rhodesii* Desmarest, 1827; *Thelazia Rhodesii* de Blainv., 1828; *Filaria bovis* Baillet, 1858; *Filaria palpebrarum* Baillet, 1858; "*Filaria lacrymalis* Gurlt" Baillet, 1866 et Railliet, 1893, pro parte.—Chez le Boeuf (*Bos taurus*) et le Buffle (*Buffelus bubalis*).

2.—*Thelazia gulosa* Railliet et Henry, 1910.—Chez le *Bos taurus*.

3.—*Thelazia alfortensis* Railliet et Henry, 1910.—Chez le *Bos taurus*.

4. *Thelazia leesei* Railliet et Henry, 1910.—Chez le *Camelus dromedarius*, dans l'humeur vitrée, dans un kyste du corps clignotant; commune sous les paupières, principalement sous le corps clignotant et dans le conduit de la glande de Harder.

5.—*Thelazia lacrymalis* (Gurlt, 1831).—Syn.: *Filaria lacrymalis* Gurlt, 1831, pro parte; *Filaria palpebralis* Wilson, 1844, non Pace, 1867; "*Filaria palpebralis* Wilson" Railliet, 1893.—Chez le cheval (*Equus caballus*). Aurait été trouvée par Busch dans l'humeur aqueuse du même animal.

6.—*Thelazia callipaeda* Railliet et Henry, 1910.—Chez le *Canis familiaris*. Paraît commune en Birmanie.

B.—Espèces parasites des Oiseaux.—Ne possèdent pas la papille impaire précloacale.

Thelazia anolabiata (Molin, 1860).—Syn.: *Spiroptera anolabiata* Molin, 1860; *Filaria anolabiata* Stossich, 1897; *Oxyspirura* ? *anolabiata* Ransom, 1904.—Chez le *Crax fasciata*, sous la nictitante et à la surface de l'oeil.

Thelazia papillosa (Molin, 1860).—Syn.: *Spiroptera papillosa* Molin, 1860; *Oxyspirura* ? *papillosa* Ransom, 1904.—Chez *Thrasactes harpyia* et *Geranospizias caerulescens*, sous la nictitante.

Thelazia campanulata (Molin, 1858).—Syn.: *Filaria campanulata* Molin, 1858.—Chez *Rupornis magnirostris*, sous la nictitante.

Thelazia ? *cirrura* (Leidy, 1886).—Syn.: *Filaria cirrura* Leidy, 1886.—Chez *Megaquiscalus major*, dans l'orbite.

Thelazia ? *stereura* (Rud., 1819).—Syn.: *Spiroptera stereura* Rud., 1819; *Oxyspirura* ? *stereura* Ransom, 1904.—Chez *Aquila maculata*, sous la nictitante et dans le méat auditif.

Genre *Ceratospira* Schneider, 1866.—Tête nue. Bouche entourée de papilles et suivie d'une courte capsule buccale.

Mâles à queue très courte, mousse, pourvue de larges ailes; de chaque côté une rangée longitudinale de papilles simples, dont 9 à II préanales. 2 spicules très inégaux,

Femelles à queue très courte, mousse. Vulve très antérieure. Parfois vivipares.

Habitat.—Cavité orbitaire des Oiseaux.

Espèce type. *C. ve siculosa* Schneider, 1866.

1.—*Ceratospira ve siculosa* Schneider, 1866.—Cavité orbitaire de l'*Ectectus pectoralis*.

2.—*Ceratospira ophthalmica* (Linstow, 1898).—Syn.: *Ancyracanthus ophthalmicus* Linstow, 1898; *Ceratospira ophthalmica* Ransom, 1904.—Cavité orbitaire du *Zonoenas brenchleyi*.

Genre *Schistorophus* n.g. (*Tetracanthus* Hemprich et Ehrenberg, 1866, non Hope, 1835; *Ancyracanthus* Schneider, 1866, pro parte, non Diesing, 1838).—Tête ornée de quatre lobes cuticulaires aigus, confondus en avant avec la cuticule, plus ou moins réunis à leur origine, surtout sur les lignes médianes, et disposés en toit. Bouche petite, généralement à deux petites lèvres ou dents. Un vestibule allongé. Œsophage composé de deux parties.

Mâles à queue mousse, arrondie, pourvue d'ailes latérales et de nombreuses papilles, les préanales disposées de chaque côté en une longue série simple. Deux spicules inégaux.

Femelles à queue courte, conique, plus ou moins obtuse; vulve dans la région postérieure ou moyenne du corps. Parfois vivipares.

Habitat.—Entre les tuniques du gésier des Oiseaux.

Espèce type: *Ancyracanthus longicornis* Hemprich et Ehrenberg, 1866.

1.—*Schistorophus longicornis* (Hemprich et Ehrenberg, 1866.—Syn.: *Ancyracanthus longicornis* Hemprich et Ehrenberg, 1866.—Entre les tuniques du gésier de *Numenius arquatus*, *Tringa variabilis*, *Totanus glottis*.

2.—*Schistorophus bicuspis* (Rud., 1819).—Syn.: *Spiroptera bicuspis* Rud., 1819; *Dispharagus bicuspis* Duj., 1845; *Histiocephalus gracilis* Dies., 1851; *Histiocephalus bicuspis* Linstow, 1878.—Entre les tuniques du gésier de *Squatarola helvetica*. Probablement identique à la forme précédente.

3.—*Schistorophus bidens* (Rud., 1819).—Syn.: *Spiroptera bidens* Rud., 1819; *Dispharagus bidens* Duj., 1845; *Spiroptera denticulata* Molin, 1860; *Ancyracanthus bidens* Schneider, 1866.—Entre les tuniques du gésier de *Merops apiaster* et peut-être d'*Astur palumbarius*.

4.—*Schistorophus laciniatus* (Molin, 1860).—Syn.: *Histiocephalus laciniatus* Molin, 1860.—Entre les tuniques du gésier de *Rallus cayennensis*.

5.—*Schistorophus* (?) *umbellifer* (Molin, 1860).—Syn.: *Spiroptera umbellifera* Molin, 1860.—Entre les tuniques du gésier d'*ibis rubra* et de *Totanus melanoleucus*.

6.—*Schistorophus* (?) *spinulosus* (Molin, 1860).—Syn.: *Filaria spinulosa* Molin, 1860.—Entre les tuniques du gésier de *Glareola austriaca*.

7.—*Schistorophus* (?) *acanthocephalicus* (Molin, 1860).—Syn.: ? *Strongylus ambiguus* Rud., 1802; ? *Spiroptera Sternae* Rud., 1819; ? *Spiroptera sternaе hirundinis* Deslongchamps, 1824; *Spiroptera acanthocephalica* Molin, 1860.—Entre les tuniques du gésier de *Sterna caspica*; peut-être dans l'oesophage de *Sterna hirundo*.

8.—*Schistorophus* (?) *capillaris* (Molin, 1860).—Syn.: *Spiroptera capillaris* Molin, 1860; *Cheilospirura capillaris* Diesing, 1861.—Entre les tuniques du gésier de *Sterna hirundo*.

Genre *Serticeps* n.g.—Tête ornée d'appendices ou festons multiples et variés. Bouche à six petites lèvres portant chacune une petite papille.

Mâles à queue obtuse; ailes caudales asymétriques; 10 paires de papilles préanales. Deux spicules très inégaux.

Femelles à queue obtuse. Vulve voisine de l'anüs.

Habitat.—Entre les tuniques du gésier des Oiseaux.

Espèce type: *Spiroptera vulvoinflata* Molin, 1860.

1.—*Serticeps vulvoinflatus* (Molin, 1860).—Syn.: *Spiroptera vulvoinflata* Molin, 1860.—Entre les tuniques du gésier de *Trochilus ochropygus*.

Genre *Cystidicola* Fischer de Waldheim, 1897.—Tête nue. Bouche circulaire suivie d'un vestibule cylindrique. Œsophage très long.

Mâles à queue arrondie à l'extrémité; ailes caudales minces; de chaque côté, une longue rangée de papilles préanales couplées et de papilles postanales simples. Deux spicules inégaux.

Femelles à queue droite, mousse. Vulve dans la région moyenne ou antérieure du corps; utérus opposés. Œufs nombreux, à coque épaisse, pourvus, au moins dans le type, de filaments polaires.

Habitat.—Vessie aérienne, plus rarement œsophage, des Poissons d'eau douce.

Espèce type: *Cystidicola farionis* Fischer, 1797.

1.—*Cystidicola farionis* Fischer, 1797.—Syn.: *Fissula cystidicola* Lamarck, 1800; *Ophiostoma cystidicola* Rud., 1801; *Spiroptera cystidicola* Rud., 1819; *Dispharagus cystidicola* Duj., 1845; *Ancyracanthus cystidicola* Schneider, 1866.—Vessie aérienne de *Trutta fario*, *Tr. trutta*, *Squalius cephalus*; vessie aérienne et œsophage de *Thymallus vulgaris*; œsophage de *Coregonus oxyrhynchus*.

2.—*Cystidicola impar* (Schneider, 1866).—Syn.: "*Gordius argillaceus* L." Martin, 1771; *Ancyracanthus impar* Schneider, 1866.—Vessie aérienne d'*Osmerus eperlanus*, *Gasterosteus aculeatus*, *Trutta fario*, *Coregonus albula*, *C. fera*, *C. lavaretus*.

3.—*Cystidicola* (?) *serrata* (Wright, 1879).—Syn.: *Ancyracanthus serratus* R. Wright, 1879.—Cœur de *Coregonus albus*.

Genre *Galeiceps* n.g.—Tête pourvue d'un renflement qui la coiffe à la façon d'un couvercle ou d'un casque. Bouche à quatre bourrelets séparés sur la surface ventrale et portant chacun à son bord interne une dent conique.

Mâles à queue obtuse ; nombreuses papilles préanales simples. Deux spicules égaux.

Femelles à queue très courte et pointue.

Habitat.—Intestin des Marsupiaux.

Espèce type: *Ancyracanthus cucullus* Linstow, 1899.

1.—*Galeiceps cucullus* (Linstow, 1899).—Syn.: *Ancyracanthus cucullus* Linstow, 1899.—Intestin de *Potamogale velox*.

Genre *Rhabdochona* n.g.—Tête nue. Bouche à deux lèvres limitant une cavité infundibuliforme soutenue par des bâtonnets longitudinaux. Œsophage de médiocre longueur, composé de deux parties distinctes.

Mâles à queue conique, pointue, recourbée ; pas d'ailes caudales ; nombreuses papilles préanales et postanales simples. Deux spicules inégaux.

Femelles à queue droite, conique, allongée. Vulve vers le tiers postérieur du corps ; utérus opposés.

Habitat.—Intestin des Poissons d'eau douce.

Espèce type: *Dispharagus denudatus* Duj., 1845.

1.—*Rhabdochona denudata* (Duj., 1845).—Syn.: ? *Fusaria cuneiformis* Zeder, 1800 ; ? *Ascaris cuneiformis* Rud., 1809 ; *Dispharagus denudatus* Duj., 1845 ; *Histiocephalus denudatus* Dies., 1851 ; *Cucullanus pachystomus* Linstow, 1873 ; ? *Dispharagus filiformis* Zschokke, 1884 ; *Ancyracanthus denudatus* Linstow, 1887 ; *Ancyracanthus denudatus* Linstow, 1902.—Intestin de nombreux Cyprinidés.

Genre *Oxyspirura* Drasche, 1897.—Tête nue, rarement avec un renflement cuticulaire. Bouche sans lèvres, suivie d'une courte capsule buccale. Queue très aigüe, oxyuriforme.

Mâles à queue généralement incurvée ou spiralée, dé pourvue d'ailes latérales ; papilles non pédonculées, les préanales en nombre assez variable (2 à 28), les postanales (1 à 8) souvent asymétriques. Deux spicules très inégaux.

Femelles à queue droite. Vulvedans la partie postérieure du corps, un peu en avant de l'anus.

Habitat.—Sous la nictitante des Oiseaux.

Espèce type: *Spiroptera cephaloptera* Molin, 1860.

1.—*Oxyspirura cephaloptera* (Molin, 1860).—Syn.: *Spiroptera cephaloptera* Molin, 1860 ; *Cheilospirura cephaloptera* Diesing, 1861 ; *Oxyspirura cephaloptera* Stossich, 1897.—Sous la nictitante de *Momotus momata* et d'*Icterus croconotus*.

2.—*Oxyspirura anacanthura* (Molin, 1860).—Syn.: *Spiroptera anacanthura* Molin, 1860 ; *Oxyspirura anacanthura* Stossich, 1897.—Sous la nictitante de *Crotophaga ani* et *Cr. major*.

3.—*Oxyspirura brevisubulata* (Molin, 1860).—Syn.: *Spiroptera brevisubulata* Molin, 1860; *Oxyspirura brevisubulata* Stossich, 1897.—Sous la nictitante d'*Otus choliba*.

4.—*Oxyspirura masoni* (Cobbold, 1879).—Syn.: *Filaria Manson* Cobbold, 1879, non Zune, 1892; *Spiroptera Emmeresi* Mégnin, 1901; *Spiroptera Manson* Marotel, 1903; *Oxyspirura Manson* Ransom, 1904.—Sous la nictitante de *Gallus domesticus*, *Meleagris gallopavo*, *Pavo cristatus domesticus*.

5.—*Oxyspirura parvorum* G. Sweet, 1910.—Syn.: *Oxyspirura parvorum* Breinl., Taylor et Johnston, 1913.—Sous la nictitante et dans la fosse lacrymo-nasale de *Gallus domesticus*.

6.—*Oxyspirura ophthalmica* (Linstow, 1903).—Syn.: *Cheilospirura ophthalmica* Linstow, 1903; *Oxyspirura ophthalmica* Ransom, 1904.—Œil de *Turnix taigoor*.

7.—*Oxyspirura siamensis* (Linstow, 1903).—Syn.: *Cheilospirura siamensis* Linstow, 1903; *Oxyspirura siamensis* Ransom, 1904.—Chez *Centropus sinensis* (probablement oeil).

8. *Oxyspirura anthochoerae* Johnston, 1911.—Syn.: *Ascaris* sp. Kreff, 1873; *Ceratospira anthochoerae* Johnston, 1911; *Oxyspirura anthochoerae* Johnston, 1912.—Œil *Anthochoera caruncalata*.

On a fait en outre rentrer dans ce genre diverses autres formes au sujet desquelles les plus grandes réserves s'imposent, par exemple :

Spiroptera sigmoidea Molin, 1860, cavité orbitaire de *Corvus frugilegus*.

Spiroptera brevipenis Molin, 1860, sous la nictitante de *Cariama cristata*.

Spiroptera heteroclita Molin, 1860, sous la nictitante de *Nothocrax urumutum*.

Spiroptera acuminata Molin, 1860, intestin de *Brycon falcatus*.

Spiroptera spiralis Molin, 1860, sous la plante des pieds des Edentés : *Bradypus cuculliger* et *Choloepus didactylus*.

SEASONAL DISTRIBUTION OF SOME ACANTHOCEPHALA FROM FRESH-WATER HOSTS *

H. J. VANCLEAVE

In a recent paper Linton (1914: 48-56) has given a brief survey of the evidence on seasonal distribution of parasites of marine fishes. He concluded this paper with the statement: "There does not appear to be evidence of any marked periodicity in the occurrence of helminth parasites of marine fishes, either adult in the alimentary canal, or immature encysted in the tissues of their hosts, beyond what may be expected where fishes are exposed to varying sources of infection in the course of their migrations." In speaking of the seasonal distribution of Acanthocephala he has recorded the occurrence of *Echinorhynchus gadi* Müller (which he called *E. acus*) in *Pseudopleuronectes americanus* ". . . in every month in which examinations were made, viz., January, February, April, May, July, August, September, October, November, and December." The mere fact that a parasite is present in its final host for the greater part of, or even for the entire, year is not proof that there is no periodicity in its occurrence. One generation of parasites might overlap another generation, yet if conditions for reinfestation were such that larvae could enter the final host only at restricted periods it would be possible to detect a periodicity in the infestation upon the basis of the distinctions between immature and mature individuals. Unfortunately most records make no mention of age of the parasites. On the other hand, if the intermediate host of the parasite constitutes a part of the food of the final host throughout the year the chances for constant reinfestation make it impossible to recognize distinct cycles of infestation.

Conditions of life in fresh-water are so much more varied than in the ocean that it would not be surprising to find seasonal changes in kinds of parasites and degrees of infestation more marked in hosts from fresh water than in hosts from the ocean. Very little has been done toward establishing any correlation between extent or degree of parasitic infestation and periodicity of occurrence. The records on these topics deal almost exclusively with the general problem of the number of parasites found in a given host without further analysis beyond an occasional tabulation of the data for the classes or orders of the parasites found. A number of writers have recorded the

* Contributions from the Zoological Laboratory of the University of Illinois, No. 58.

months in which they have found *Acanthocephala* in various hosts without furnishing any data on the presence or absence of the same parasites in the same hosts at other times of the year. Thus Zschokke (1884: 58) has recorded *Pomphorhynchus laevis* (Müller) from various fishes from January to June, but he has not given any evidence or proof of its absence for the remainder of the year. In fact his records seem only to indicate the dates when he chanced to examine fish which were infested with *P. laevis* rather than to represent an attempt on his part to establish the limits of the seasons when this parasite occurs in its final host.

In collecting fresh-water *Acanthocephala* the writer has been impressed by the varying degrees of infestation of certain hosts at different times of the year. A search of the literature has furnished so little actual information upon this subject that it seemed worth while to investigate the question, especially since Linton has rather summarily dismissed the topic with a brief generalization. The records from which the following data have been gathered comprise three species of *Acanthocephala*. In one of these no marked seasonal distribution is evident, while in the other two definite seasonal cycles mark the occurrence of the parasite in its final host.

Neoechinorhynchus emydis* (Leidy) occurs in the intestine of a number of fresh-water turtles. The records of the writer and of Mr. H. W. Stunkard include the examination of over 200 individuals belonging to species susceptible to infestation with *N. emydis*. These came from Iowa, Ohio, West Virginia, Texas, and various points in Illinois. The unselected data from all the records when assembled and tabulated presented evidence of a seasonal distribution of *N. emydis*, which upon closer examination of the data proved to be spurious. Parasites of this species were recorded from hosts examined in October, November, December, January, and February with a few records of occurrence in July. Records of examinations in April, June, and September showed no infestation with this parasite. This in itself seemed to indicate a restriction of *N. emydis* in the intestine of its final host to a limited portion of the year. However, further examination revealed that certain localities within the geographical range of the species are free from that parasite. By a strange coincidence turtles happened to be examined from these localities in months when no records were available for regions where *N. emydis* is known to occur. This shows how statistical data if not carefully checked may give false evidence of cyclic occurrence of an organism.

* *Neoechinorhynchus*, Stiles and Hassall, 1905=*Eorhynchus*, VanC., 1914=*Neorhynchus*, Hamann, 1892, preoccupied.

Indirect evidence has shown that *N. emydis* occurs in the intestine of its final host throughout the year. Turtles from Havana, Ill., have been kept without food in aquaria fed by the University of Illinois water supply, which is from deep wells, for about eleven months. At the end of this time one turtle still harbored twelve mature specimens of *N. emydis* in its intestine. The evidence of an original infestation lasting practically a year, together with the fact that in many instances the writer has found fully mature, immature, and intermediate specimens in the intestine of the same individual proves that turtles in regions where *N. emydis* occurs are constantly exposed to reinfestation with that species. Consequently there is no cyclic change in the degree of infestation from month to month.

It is interesting to note that while *N. emydis* has a broad geographical distribution, occurring in the records under consideration at certain points in Illinois, North Carolina, and Texas, it is by no means generally distributed over its range. In Illinois, for example, turtles of species susceptible to infestation with *N. emydis* have been collected at Urbana, Muncie, and Chicago, and in no case has a single specimen of *N. emydis* been found. It seems strange that a species with such a broad dispersal should not have followed the dispersal of its final host. This probably finds explanation upon the grounds that in the localities where the parasite does not now occur if it was originally or subsequently introduced the embryos when expelled from the intestine of the final host were not taken up by animals in which the larvae could develop or in case they did find lodging in a host it must have been in some animal which was not used by the turtles as food. Thus through the lack of adaptability to new conditions brought on by the specialization accompanying parasitism this species has been excluded from some regions which are included within its limits of distribution.

In contrast with the lack of periodicity in the species just discussed may be noted the condition found in *Neoechinorhynchus gracilisensis* (VanC.) found in the intestine and intestinal caeca of the gizzard-shad, *Dorosoma cepedianum* (LeSueur), from the Illinois River system. During the period from 1909 to 1912 the writer examined more than 300 gizzard-shad for parasites. But two species of parasites have been found. Both of these were Acanthocephala belonging to the genus *Neoechinorhynchus*. *N. gracilisensis* has been found in October, November, December, February, March, and April, but specimens at these different dates displayed wide variation in degree of sexual maturity. Those collected in October were almost invariably small and immature, with a high percentage of infestation. By the latter part of November individuals of this species had reached full sexual maturity, as indicated by the numbers of hard-shelled embryos contained in the body cavities of the females. In April the percentage

of infestation had decreased to less than one half of that found for October, and the number of individuals per host also had decreased though every parasite had reached full sexual maturity and the maximum size for the species. Numerous examinations in the months of June, July, and August have failed to give even a single specimen of this species. From the foregoing data it is evident that the introduction of *N. gracilisensis* into the final host must occur in early fall, probably in September. The individuals have become fully mature by April and disappear entirely from the final host during the months of June, July, and August. In an earlier paper (VanCleave 1913; 181) I have indicated the probable relationship between this periodicity of infestation and the food habits of the gizzard-shad. Observations upon the stomach contents of the shad, which is primarily a scavenger, have failed to throw any light upon the probable intermediate host of this parasite. The entire digestive tract is usually filled with mud and decomposed plant tissues with a very few shelled rhizopods and some species of microcrustacea.

TABLE SHOWING SEASONAL DISTRIBUTION OF THREE FRESH-WATER SPECIES OF NEOECHINORHYNCHUS

Species	January	February	March	April	May	June	July	August	September	October	November	December
<i>N. emydis</i>	+	+	X	X	X	X	+	X	X	+	+	+
<i>N. gracilisensis</i>	X	+	+	+	±	—	—	—	±	+	+	+
<i>N. longirostris</i>	—	—	—	—	±	+	+	+	X	X	+	+

* For additional experimental evidence see text.

+ Positive records of occurrence based upon examination of hosts.

— Absence from all hosts examined.

± Extremely probable occurrence. Though definite records of infestation are wanting the stage of maturity of individuals collected the preceding or the following month indicates that a complete gradation of stages in development necessitates an overlapping of infestation into adjacent month.

X records, both positive and negative, wanting though stages of maturity of the parasites in the two adjacent months together with the data upon longevity of the species in the final host justifies the assumption of a positive infestation.

Neoechinorhynchus longirostris (VanC.), the second species found in the intestine and intestinal caeca of the shad, occurs in much smaller numbers and in but a very small percentage of fishes examined. Immature individuals were found in June and July. Gravid females were found in August, November, and December. While the number of records is insufficient to permit of establishing all points in a seasonal cycle, yet the evidence at hand indicates that the host is free from parasites of this species from late winter until early summer.

In the case of both species of *Neoechinorhynchus* from the gizzard-shad the relatively short life in the body of the final host is noticeable.

Moreover, the parasites of a given species collected at the same time from a given region have all reached approximately the same stage in development. This indicates that the period when infestation may occur is very brief. Attention should also be called to the fact that periods of infestation in these two species are not coexistent.

CONCLUSIONS

1. Seasonal distribution of fresh-water *Acanthocephala* varies in different species. No general statement can be made to apply to the entire group.

2. *Neoechinorhynchus emydis* (Leidy) has broad limits of geographical distribution, but has never been found in turtles of susceptible species from some localities within its range of distribution.

3. *N. emydis* occurs in turtles from some localities at all seasons of the year.

4. The same host may harbor specimens of *N. emydis* in all stages of development between immature and fully mature. This shows the host must be exposed constantly to sources of infestation.

5. There is no cyclic change in the degree of infestation with this species from month to month.

6. *N. gracilisensis* (VanC.) enters the gizzard-shad in early fall, probably September; in April or May it attains sexual maturity and is finally expelled. During the summer the gizzard-shad is not parasitized by this species.

7. *N. longirostris* (VanC.) parasitizes the gizzard-shad in the summer, reaches full sexual maturity by midwinter, and disappears entirely from spring to early summer.

8. The demonstrable presence of a seasonal cycle in the life history of a parasite involving two or more hosts is dependent upon (a) longevity of the parasite in the final host; (b) extent of the time in which infestation of the final host may occur; (c) length of time required for development of the larva in the intermediate host; (d) seasonal changes in the food habits of the final host, or active migrations of the host to and from sources of infestation.

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ON THE INTERMEDIATE HOSTS OF THE LUNG DISTOME.
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Japan is famous for being considerably infested with lung distome. It is about thirty-four years since the human lung distome was discovered by Kiyono, Yamagata, Nakahama, and Suga in Okayama prefecture, near the center of the country. During these years though numerous reports on symptoms of patients and on diagnosis and many pathological notes have been published by investigators from various districts of the country, the result of the developmental investigations has not yet been achieved, nothing being known of a life history of the parasite. Fortunately, however, light has been thrown upon the subject by the recent discovery of an intermediate, probably the second, host of this worm by Koan Nakagawa, director of the Shinchiku Hospital, Formosa, who has been earnestly investigating this parasite in Formosa for several years.

I have also studied experimentally the life-history of this worm, and found certain species of fresh water crabs as intermediate hosts. I shall here communicate the results obtained from my experiments.

INTERMEDIATE HOST

In Formosa Nakagawa found the encysted larvae in two fresh water crabs and experimentally proved that they grew up to the lung distomes. The two crabs were identified by A. Terao as follows: *Potamon* (*Geothelphusa*) *obtusipes* (Stimpson), *P. (Ge.) dehaanii* (White).

Nakagawa added that a fresh-water crab *Eriocheir japonicus* (De Haan) will also probably prove to be the intermediate host.

I have experimentally proved that the encysted larvae of this worm are found in three species of fresh-water crabs from various districts of Japan proper. They are identified as follows: 1. *Patamon dehaanii* (White); 2. *Sesarma dehaanii* (Milne Edwards); 3. *Eriocheir japonicus* (De Haan).

These species can be found in any part of Japan. *P. dehaanii* is small in size and light reddish brown or purple in color. It is a common crab in the shallow water of a mountain stream. This crab is edible and used for food commonly in some districts and rarely in other parts of this county. It can be eaten raw or cooked. *E. japon-*

icus is large in size and dark brown or black in color, very common in any brook and river of Japan, including Formosa and Corea. Large hairy forceps are characteristic of this species. It also is edible and commonly used for food in all districts, though generally eaten cooked being roasted, boiled, or fried. *S. dehaani* is of median size, the same in color as *E. japonicus*, having light reddish purple forceps. It lives generally in the lower parts of a river in various parts of our country. This species is not used for food.

Distribution of Encysted Larvae in Body of Intermediate Hosts.—The encysted larvae occur generally in the liver, muscles, and gills of the host. The distribution of the encysted larvae varies but slightly according to the species of the host, so far as I have examined. In *P. dehaanii* and *P. obtusipes*, they are found frequently in the liver, and rarely in muscles and gills; in *E. japonicus* chiefly in gills, muscles, and hypodermis, and rarely in the liver; and in *S. dehaani* mainly in the liver and very rarely in the gills. In the liver the encysted larvae are attached loosely to the lobes of the organ so that they may be easily detached. In the gills they adhere between the lamellae in the case of *P. dehaanii*, but are found only in the blood vessel running through the median line of the upper surface of the gill in the case of *japonicus*. In the latter species they occur not only in the muscles of the trunk, but in muscle and hypodermis of all appendages. The encysted larvae in the muscles and hypodermis or in the blood vessels are easily movable.

Frequency of Occurrence and Number of Encysted Larvae in Host.—It is reported by Nakagawa that about 100 per cent. of *P. obtusipes* are infected with the encysted larvae at Shinchiku, the most famous district for the lung distome in Formosa. Ryo Ando reported that about 40 to 70 per cent. of *P. dehaanii* of Gifu prefecture are infected with the larvae. According to my own examination *E. japonicus* of Tokushima and Okayama prefectures is infected to the extent of about 70 to 85 per cent., and *S. dehaani* of Osaka prefecture to about 20 to 80 per cent. The number of the cysts in one crab varies considerably according to species of the host, and even in the same species it varies according to locality and other conditions. In my own examinations I found some *E. japonicus* infected with several hundreds of the encysted larvae while others were infected only with a few in spite of the locality being the same. *S. dehaani* was generally infected with 2 to 30 cysts of the larvae. In *P. dehaanii* from Okayama and Nigigsta prefectures I found only a few cysts while Ando is reported to have obtained several hundreds of cysts in the same species from Gifu prefecture.

I give here tables showing the percentages of the infected crabs and the numbers of the cysts in the hosts examined by myself.

TABLE 1.—*S. DEHAANI* FROM HIEJIMA, OSAKA PREFECTURE

Date	Number of Crabs Examined	Number of Crabs Infected with Cysts	Percentage of Infected Crabs	Maximum of Cysts in One Crab	Minimum of Cysts in One Crab	Total Number of Cysts in Crabs Infected	Average Number of Cysts in One Crab	Number
10/VI	19	4	21.05%	3	1	7	1.43	1
11/VI	8	2	25.00%	2	2	4	2.00	2
12/VI	3	1	33.33%	2	2	2	2.00	3
14/VI	20	2	10.00%	2	1	3	1.50	4
15/VI	12	2	16.66%	3	1	4	2.00	5
16/VI	12	1	8.33%	1	1	1	1.00	6
17/VI	21	5	23.80%	2	1	6	1.20	7
18/VI	8	3	37.50%	4	1	7	2.33	8
22/VI	24	2	8.33%	2	1	3	1.50	9
28/VI	9	2	22.22%	3	2	5	2.50	10
10/VII	10	2	20.00%	8	2	10	5.00	11
11/VII	9	2	22.22%	7	3	10	5.00	12
12/VII	1	1	100.00%	1	1	1	1.00	13
13/VII	11	3	27.27%	3	1	6	2.00	14
14/VII	9	4	44.44%	4	1	10	2.50	15
15/VII	16	2	12.50%	2	1	3	1.50	16
16/VII	8	2	25.00%	3	2	5	2.50	17
	260	40	20.00%	8	1	87	2.17	17

TABLE 2.—*S. DEHAANI* FROM EBIE, OZAKA PREFECTURE

Date	Number of Crabs Examined	Number of Crabs Infected with Cysts	Percentage of Infected Crabs	Maximum of Cysts in One Crab	Minimum of Cysts in One Crab	Total Number of Cysts in Crabs Infected	Average Number of Cysts in One Crab	Number
28/VIII	5	4	80.0%	29	9	89	22.5	1
30/VIII	2	2	100.0%	26	26	52	26.0	2
1/IX	6	5	83.3%	30	15	107	21.4	3
2/IX	5	5	100.0%	22	1	49	9.8	4
	18	16	88.8%	20	1	297	18.5	4

TABLE 3.—*E. JAPONICUS* FROM IKUINA, TOKUSHIMA PREFECTURE

Date	Number of Crabs Examined	Number of Crabs Infected with Cysts	Percentage of Infected Crabs	Maximum of Cysts in One Crab	Minimum of Cysts in One Crab	Total Number of Cysts in Crabs Infected	Average Number of Cysts in One Crab	Number
23/VII	10	9	90 %	19	3	96	10.66	1
25/VII	4	4	100 %	26	1	56	14.00	2
26/VII	7	7	100 %	14	1	29	4.14	3
27/VII	15	13	86.6%	12	1	61	4.69	4
28/VII	1	1	100 %	86	86	86	86.00	5
2/VIII	10	3	33.3%	77	7	161	59.66	6
3/VIII	15	7	46.6%	40	1	70	10.00	7
3/VIII	5	3	60.0%	8
6/VIII	8	6	75 %	48	2	85	14.16	9
7/VIII	2	1	50 %	343	343	343	343.00	10
	77	54	70.1%	343	1	987	19.35	10

TABLE 4.—DISTRIBUTION OF CYSTS IN THE BODY OF NO. 10 IN TABLE 3

Gills on both sides.....	51
Body muscles on left side.....	99
Body muscles on right side.....	169
Forceps on right side.....	19
Third leg on right side.....	12
Third leg on left side.....	23

TABLE 5.—DISTRIBUTION OF CYSTS IN THE LEGS OF THE CRAB IN THE PRECEDING TABLE

	Ischiopodite Meropodite	Carpopodite	Propodite	Dactylopodite	Total
Right forceps.....	4	12	3	0	19
Right third leg.....	7	3	2	0	12
Left third leg.....	13	6	4	0	23
Total.....	24	21	9	0	54

TABLE 6.—NINE CYSTS IN GILLS, FIFTY-FIVE IN MUSCLES AND HYPODERMIS

	First Legs (Forceps)		Second Legs		Third Legs		Fourth Legs		Fifth Legs	
	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left
Muscles attached.....	1	2	2	1	6	4	0	2	1	1
Basipodite.....	1	0	2	1	0	1	0	1	0	0
Ischiopodite.....	1	3	0	2	0	2	1	0	0	2
Meropodite.....	4	3	0	0	2	0	0	2	1	1
Carpopodite.....	0	1	0	0	0	1	2	1	0	0
Propodite.....	0	0	0	0	0	0	0	0	0	0
Dactylopodite.....	0	0	0	0	0	0	0	0	0	0
Total.....	7	9	4	4	8	8	3	6	2	4

TABLE 7.—DISTRIBUTION OF CYSTS IN *E. JAPONICUS* FROM TOMIOKA, TOKUSHIMA PREFECTURE

Number	Total Number of Cysts	Cysts in Gills	Cysts in Muscles, etc.	Number	Total Number of Cysts	Cysts in Gills	Cysts in Muscles, etc.
1	7	7	...	16	106	106	...
2	5	5	...	17
3	5	5	...	18
4	98	31	64*	19
5	193	69	124	20	21	21	...
6	159	67	92	21	11	11	...
7	40	8	32	22	36	36	...
8	2	2	..	23	48	48	...
9	161	40	121	24	16	16	...
10	90	20	70	25	22	22	...
11	3	3	..	26
12	11	11	..	27	35	35	...
13	76	24	52	28	12	12	...
14	51	33	18	29	53	53	...
15	17	17	..	30	390	133	257

* Three in liver.

TABLE 8.—FROM THE ABOVE TABLE

Number of crabs examined.....	30
Number of crabs infected with cysts.....	26
Percentage of infected crabs.....	86.6%
Total number of cysts.....	1,068
Average number of cysts in one crab.....	64.1

TABLE 9.—DISTRIBUTION OF CYSTS IN THE MUSCLES AND THE HYPODERMIS OF ONE *E. JAPONICUS* HAVING 390 CYSTS IN ALL (NO. 30 OF TABLE 7)

	First Leg (Forceps)	Second Leg	Third Leg	Fourth Leg	Fifth Leg	Total
Body muscles attached to	49	28	28	9	12	126
Ischiopodite.....	..	4	0	2	0	6
Meropodite.....	10	11	16	16	22	75
Carpopodite.....	10	3	9	6	0	28
Propodite.....	9	2	6	3	2	22
Dactylopodite.....	0	0	0	0	0	0
Total.....	78	48	59	36	36	257

Morphology of the Encysted Larvae.—The encysted larvae found in the above named crabs are almost spherical or rarely elliptical in shape, measuring from 0.25 to 0.55 mm. in diameter. The fully grown cysts vary between 0.30 and 0.55 mm. The wall of the cyst is a transparent chitinous membrane of tolerable thickness. In the fully developed larva in a cyst one may easily and distinctly recognize the organs, for example, both oral and ventral suckers, and alimentary tract including pharynx, esophagus, intestinal coeca, and excretory vesicle. The oral and ventral suckers are of nearly equal size, the former is distinctly visible when the worm is moving and the latter becomes somewhat indistinct on account of being obstructed by an extension of the excretory vesicle. The pharynx is small, the esophagus short and the intestinal coeca run posteriad in a strongly winding course along both sides of body, ending blindly at or near the posterior end. The excretory vesicle occupies nearly all the space between the intestinal coeca.

The parenchymous tissue of the body is tinged with light red pigment so that a cyst containing a larva is easily recognizable even in liver or in muscle. This pigmentation is very convenient in searching for the cyst. The young worm just out of a cyst is generally elongated oval in shape. Under a slight pressure one may more clearly observe all the internal organs, the minute spines with which the body surface is provided and the excretory vesicle contracted in discharging its contents.

Animal Experiments with Encysted Larvae.—In the feeding experiments I have used young cats and dogs. These animals were all brought from uninfested districts and were carefully examined that none of them was infected with lung distomes. Some of them have already died or been killed and used for study of the development of the young worms in these hosts, but the others are yet at the present under experimentation. I will here describe the results of some of my experiments.

(A) Young cat. The animal was fed with 20 cysts on July 26, 80 on the 28th, and 130 on August 2. These cysts were all taken from the gills of *E. japonicus* collected from Tokushima prefecture. The cat died August 10, having passed 16, 14 and 9 days after the first, second and third feedings, respectively. Before her death she was extremely anemic and atrophied. In dissection the next day, numerous cestode larvae were found in the body wall as well as in the body cavities, abdominal and pleural. Some portions of the body wall occupied by the worms had suppurated. It was easy to believe that the anemic and atrophic symptoms and consequently the death of the cat were caused by the presence of these larval cestodes.

In the abdominal cavity I found 18 young worms floating in serous fluid and adhering to the omentum, mesentery, and inner surface of the abdominal body wall, and in the pleural cavity 16 worms in serous fluid and on the pleural membrane. These young distomes in the body cavity were all nearly equal in size, measuring from 1 to 2 mm. in length in compressed specimens. The lungs were not yet occupied by the worms.

(B) Young cat. Fed with 80 cysts August 7 and killed on 17th of the same month, 11 days after feeding. All the cysts were collected from the same locality given in the preceding example. I found 5 young worms in the abdominal cavity and 6 in the pleural cavity. These worms measure about 1 mm. in length and 0.5 mm. in breadth. (Figure 6.)

(C) Young dog. Fed with 33 cysts August 14, 46 on 17th, 90 on 28th, 50 on 30th, and 32 on September 1. The cysts of the first and the second feedings were obtained from *E. japonicus* of Tokushima prefecture and all the remaining cysts were taken from *S. dehaani* of Ebie, a suburb of Osaka. The dog died September 29, having passed 46, 43, 32, 30, and 29 days after each corresponding feeding. Some inflammations were observed here and there in the inner surface of the intestinal wall. This seemed probably to have been caused by the action of the young worms of the lung distomes. I found also a great number of *Dipylidium caninum* and a few *Ankylostomum caninum* in the small intestine. Omentum and the mesentery were slightly congested here and there. There were numbers of perforations on the inner surface of the abdominal body wall. Around the perforations more or less hemorrhage was observed between the muscular layers of the abdominal wall. These perforations were evidently produced by the young worms which were found everywhere in the serous fluid or on the surface of various organs in the abdominal cavity where I had obtained 30 young worms. A number of perforations was observable on the diaphragm through which the young worms seem to have passed from the abdominal to the pleural cavity.

In the pleural cavity 43 young worms were obtained in the serous fluid and on the surface of the various organs: lungs, heart, and pleural membrane. The size and the shape of the worms in the body cavities were extremely variable according to the state of contraction. In fixed specimens, the length varies from 1.5 to 5 mm. and the breadth from 0.5 to 2 mm. I found a good specimen which is most favorable to demonstrate that the worm in the pleural cavity enters the lungs from its surface by perforating. Numerous worms in various stages of development were observed in the lungs. The worms of large size

in the lungs were plainly observable from the outside or easily felt by touching on the surface of lungs.

(D) Young cat. Fed with 4 cysts June 15, 1 on 16, and 3 on 17, and was killed on August 17. It was 62 to 64 days after the feedings. All the cysts were taken from the liver of *S. dehaani*. In the dissection of this animal I found 2 young distomes, one in the pleural cavity, the other in the right lung. The dimensions of this worm were about 4 mm. long and 2 mm. broad. The worm in the lung was not yet matured.

From my observations in the above experiments, I have learned the general course which the young worms travel from the intestine to the lungs of the host. Briefly stated, it is as follows:

The encysted larvae swallowed by a host come out of their cysts in the stomach or intestine through the action of gastric or intestinal juice. The larvae coming out of the cysts are actively mobile. They pierce the wall of the intestine. They stay for some time in the abdominal cavity and wander about here and there; thence they pierce through the diaphragm to enter the pleural cavity. Here again they stay for some time and finally penetrate into the lungs from their surface.

In addition to this general course, the young distomes in the abdominal cavity may also penetrate the abdominal wall and move around in the muscular layers of the connective tissues, as stated above in Case C. Some worms in the pleural cavity may proceed cephalad, taking their course through the loose connective tissues along the esophagus or the blood vessels. Thus the young worm of the lung distome has evidently a wandering power by which it can pass through the muscular and connective tissues. It is obvious that the discovery of the wandering character of the young worm throws light on the accounts of cerebral and spinal paragonimiasis which are attributed to the lung distomes.

October, 1915.

EXPLANATION OF PLATE

Fig. 1.—Cyst attached to lobule of liver of *S. dehaani*. x60.

Fig. 2.—Longitudinal section of the right third leg of *E. japonicus*, showing distribution of cysts. Natural size; b, basipodite; c, carpopodite; d, dactylopodite; g, gill; h, hypodermis; i, ischiopodite; k, muscle; l, encysted larvae; p, propodite.

Fig. 3.—Cyst from the gill of *E. japonicus*. x60; i, intestinal coeca; e, excretory vesicle; o, oral sucker; v, ventral sucker.

Fig. 4.—Larva escaping from cyst. x60.

Fig. 5.—Larva coming out of cyst; slightly compressed specimen. x60.

Fig. 6.—Young worm in abdominal cavity of young cat. x60; c, excretory canal; note also intestinal coeca.

PLATE



Fig. 1

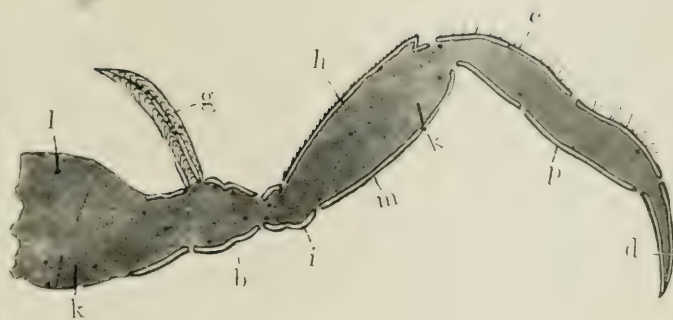


Fig. 2



Fig. 3

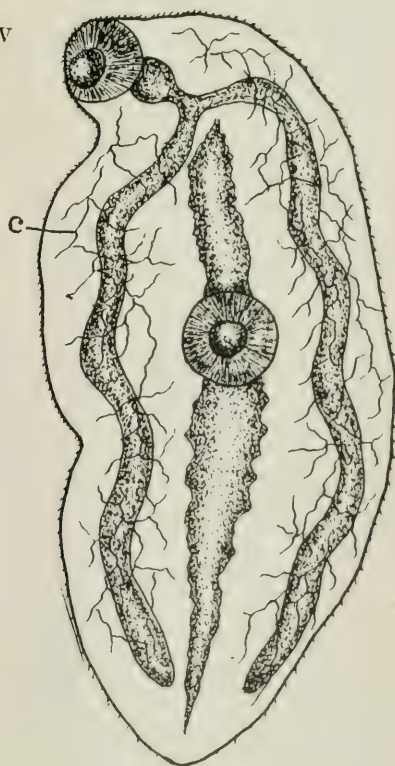


Fig. 6



Fig. 4



Fig. 5

GONGYLONEMA IN THE ROLE OF A HUMAN PARASITE *

HENRY B. WARD

Through the kindness of Dean Charles E. Brookover of the University of Arkansas Medical Department, Little Rock, I received recently a specimen of a nematode which has not heretofore been recorded as a human parasite. The worm was removed from the lip of a girl. The conditions surrounding the case are reported as follows by the attending physician, Dr. Robert Lee Covington of Jefferson, Ark., to whom I am indebted not only for the case history cited here, but also for replies to several inquiries on the matter necessary to perfect the record for publication. Dr. Covington's report is as follows:

Miss —, 16 years old. I was called to see her September 3. Found her suffering with considerable digestive disturbance, vomiting continuously for two or three hours; temperature 101.5 F. She felt chilly. I administered calomel followed with castor oil. Later I gave her full doses of turpentine and castor oil. The fever would come and go and for this I gave quinin. She improved some; later the fever abated entirely, but some digestive disturbance still persisted. She was very anemic and irritable. September 12 I dismissed her.

I was again called October 1. She wanted me to see what that was running around under the skin of her lip, as she expressed it. I thought it was pure imagination. She said she could see it by looking in the mirror; she said it looked like a worm, and at times she could feel it leave her lips and go back as far as the fauces. I examined the lower lip where she said it had stopped. I discovered the outline of what looked like a worm about three quarters of an inch in length and about the size of a No. 60 sewing thread; it was just beneath the mucous membrane. I inserted a needle under it and pulled out a little of one end, but before I could grasp it, it got away, moving back near the corner of the mouth. There I ran the needle under it midway between the ends and pulled upward, bringing a small loop through the mucous membrane. It held on so tight that the ends on each side of the needle cut their way out.

Up to this time she was extremely anemic and very cross and irritable, but after the removal of the worm she rapidly improved, her disposition changed, and now she is not like the same girl. She seems to be sound and well at this date.

DR. ROBERT LEE COVINGTON,
Jefferson, Ark.

Jan. 26, 1916.

In response to specific questions Dr. Covington stated that the worm moved up and down in the tissues three or four times, extending its migrations from the lips at least as far back as the fauces. It was actually seen by the patient three times and she reported it positively

* Contributions from the Zoological Laboratory of the University of Illinois, No. 59.

to her father the day before its removal. It could be discerned clearly enough to tell that it was a worm; in color it was a little lighter than the mucous membrane. During its movements the worm was definitely sensible to the patient only when it approached the skin and it was not seen or felt in this organ anywhere except on the inside of the lips. It was seen only inside the mouth through the thin mucous membrane of that cavity and so far as known did not approach the external skin save at the inner border of the lower lip.

From this record one may safely conclude that it migrated to and fro through the loose connective tissue beneath the oral mucosa and endeavored to move up into the firmer derma only when it approached the lips. From the fact that it evaded the surgeon for a time, one can see that the movement was free and active.

Subsequently Dr. Covington wrote regarding the patient: "She has lived here all her life, has not traveled in any other country. . . . Sanitary conditions are not just what they ought to be. They get drinking water from a well that is very shallow and fills up to the top when it rains heavily."

On preliminary examination it appeared that the specimen had suffered somewhat in the handling incidental to its forcible removal, and was not in such histological condition that much could be said definitely regarding its internal structure.

Under a dissecting lens the specimen (Fig. 1) is seen to be a nematode worm, light brown in color, semitranslucent, and loosely coiled, though both ends are nearly straight. The worm measures 42.1 mm. in total length and is of nearly equal diameter throughout, tapering only a little near the two ends which terminate rather abruptly.

The anterior end appears to taper more toward the bluntly round tip and for a space of 1.4 mm. from the end the surface is ornamented by various cuticular outgrowths like scales or tubercles. These are arranged in somewhat definite fashion (Fig. 2). A cuticular ridge extends along the lateral line of the body starting at a point about 0.25 mm. from the extreme anterior tip and running back about 1.5 mm. In the anterior region this ridge is slightly irregular but nearly equal in height at all points; further back it is divided by deep indentations into long ovals. At the place where it starts the diameter of the body is about 0.1 mm. and at its posterior end the body has increased in diameter to 0.19 mm.

In the submedian lines are rows of scales, plates, or tubercles that start just behind the front end of the ridge just described and extend backwards only about 1.1 mm. in definite and regular order. These tubercles stand in a linear series so close together that they are practically continuous even though separated from each other by a distinct boundary line. They vary in form and size but on the average

are nearly square in profile and measure 0.022 mm. in height and 0.025 to 0.3 or rarely 0.045 mm. in length. In front of this close-set series and of the cuticular ridge one finds a number of isolated cuticular bosses which in part line up with the ridge or the series and in part do not. Between the ridge and the series are a few isolated scales. These are irregular, detached, and on the average larger than the tubercles in the regular series.

The anterior tip of the body possesses a small infundibuliform crown surrounding the mouth which may represent a group of lips with some papillae. From the orifice a capilliform esophagus extends posteriad and disappears from view behind the anterior tip of the cuticular ridge. In the mid-ventral line about 0.6 mm. from the anterior tip is a peculiar papilla that probably surrounds the excretory pore. The location of the end of the esophagus is difficult to determine precisely; it appears to be about 7.5 mm. from the anterior end. Here the diameter of the body is 0.23 mm.; a measure which is maintained with only slight variations from 0.22 to 0.24 mm. approximately, throughout the entire length until the caudal region is attained.

The vulva is a ventral, slightly prominent pore with raised lips, located 2.15 mm. from the posterior tip of the body. Even behind it the body retains its uniform caliber of approximately 0.23 mm. until close before the anus or about 0.25 mm. from the exterior tip. This region is a little damaged by handling, but shows distinctly a slight concavity on the dorsal surface making the bluntly rounded posterior tip of the body turn a bit upwards. At the anal orifice the body measures 0.09 mm. in diameter, which is evidently somewhat less than normal owing to the injury already noted. The taper begins only a short distance anterior to the anus, and if the specimen had been uninjured, would have been regular apparently from that point to the rounded tip. There is certainly no abrupt change in diameter either before or behind the anus.

There can be no doubt that this specimen belongs to the genus *Gongylonema* in the family of the Filariidae. *Gongylonema* is a nematode peculiar in habit in that it forms a sinuous gallery in the mucous epithelium of the esophagus. The worm lies in this tunnel with the anterior region alone projecting from it. With a single exception all species are found in mammals. The body is very long and thread-like, showing a slight taper toward both extremities. The characteristic feature of this genus is the presence on the anterior region of a considerable number of scales or tubercles which are elevated thickenings of the external cuticula only. These differ in size, number, and arrangement in different species. Cuticular folds stand out from the surface along the median or lateral lines in the

anterior region, and form conspicuous features in the external aspect of the worm. These also vary in number, form, and extent in various species. The vulva is located near the posterior end, and its precise position as well as the form and size of the tip of the tail are valuable in determining the species of female specimens.

Two species of *Gongylonema* are common to domestic animals in the United States, and fall under suspicion as possible occasional parasites of man. These are *G. scutatum* and *G. pulchrum*. Of *G. ingluvicola* Ransom from chickens it need only be said that the structure of the adult is too dissimilar to allow of the surmise that this specimen is an erratic individual of that species. But the other two are much alike in structure and are abundant in some parts of this country, if not in most regions; they are also both very similar to the specimen under consideration.

G. scutatum was recently described with some care by Ransom (1911) who declares that it is very common in this country and can be found in a large percentage of cattle and sheep slaughtered at abattoirs.

G. pulchrum is known as a parasite of the hog in Europe, and has been found also in North Africa by Seurat (1912). It does not seem to have been reported in print from the United States previous to this year when it was briefly mentioned by Ransom and Hall (1916) in connection with the discussion of experiments on the life history of *G. scutatum*. They have found it to be frequent in the vicinity of Washington, D. C., and it doubtless occurs abundantly in the pig in other regions as well. Dr. B. H. Ransom was good enough to send me specimens for comparison. These came from the collections of the Bureau of Animal Industry and were taken from a hog at Bethesda, Md., in January, 1914.

These two species are very similar, and in the present case it is impossible to assert positively which one is the specimen under consideration. It agrees in length with *G. pulchrum* and as nearly as can be ascertained also in the character of the female genital organs of which a comparative study has been published by Seurat (1912). The caudal tip is also slightly concave on the dorsum as has been described in that species. However, this specimen is a trifle over ordinary length for *G. pulchrum*, although it has not yet reached full sexual maturity. But the genital pore is salient, although not notably so, a feature which is said to be characteristic of *G. pulchrum* though wanting in *G. scutatum*. On the whole, I am inclined to determine the specimen from man as *G. pulchrum*. The common ascarid of man, the stomach worm, *A. lumbricoides*, is also a parasite of the pig in which it occurs abundantly in all parts of the country.

The specimen under consideration manifested a habit that so far as known is not characteristic of the genus to which it must be assigned. It was engaged in active migrations through the subdermal connective tissue, and the patient was conscious of the fact. It had approached near enough to the surface to be seen and correctly diagnosed in form, and had wandered away into deeper tissues. The worm may have been thus active over a period of a month or less. It moved through the connective tissue rapidly enough to render its capture and removal a matter of skill and dexterity. Such performances are not reported in other accounts of the genus, which as already noted is found in the mucosa of the esophagus in its normal host, and has not been collected from other organs or regions. The presence of the chicken species in the wall of the crop is not a real departure from this general habit for it occurs there in the mucous lining according to Ransom's report. It is, of course, possible that these forms regularly inhabit the subdermal connective tissue during a period of their life cycle and appear in the esophageal mucosa at the time of sexual maturity; or such an occurrence may be exceptional but actual in the normal host also. In that event it would readily escape notice under usual conditions. There is no basis for deciding whether such wanderings are usual but unnoted heretofore in the normal host, so rare in it as to have evaded observation thus far, or peculiar to the human host or to this particular case.

The habit recalls very strikingly the wandering through subdermal tissues of the African eyeworm, *Filaria loa*, which has received its name from its frequent appearance in the subcutaneous connective tissues near the eyeball. It is also well known to occur in the same tissues elsewhere in the body. Among the cases on record are a few which report its removal from the lip or near the same, but it occurs habitually near the external skin and is not reported from the vicinity of the mucosa. Yet it is not at all impossible that in some instance where no careful examination was made a specimen of Gongylonema has been interpreted as the loa. The length of the specimen reported here and its appearance agree in general with the loa, and while it is not quite so heavy nor so opaque, the two might readily be confused if no careful microscopical examination were made; all the more so since the loa is the only nematode which has been known to move about in subdermal tissues and that striking habit would have been taken to indicate the type of parasite at hand.

There are other records of parasitic nematodes from man in this country that should be brought briefly under consideration in connection with the case in hand. In 1850 Dr. Joseph Leidy of Philadelphia

described a human parasite as *Filaria hominis oris* from a specimen in the collection of the Philadelphia Academy labeled "obtained from the mouth of a child."

This account of Leidy has always been difficult to interpret. His conjecture that it was a young *Dracunculus* or Medina worm has been generally rejected and other suggestions are not entirely satisfactory. The location suggests that the specimen may have been a *Gongylonema*, not the species here reported for that is much too short, but *G. scutatum* of cattle and sheep which is of the right dimensions. Leidy was a very accurate observer and it is improbable that he would have overlooked the cuticular tubercles on the anterior end if such had been present. The condition of the specimen might have been such as to prevent the diagnosis of these structures, but on the whole the case can hardly be assigned to *Gongylonema* even tentatively.

The source of the infection in the case at hand can not be positively ascertained and yet recent studies suggest a very probable explanation. Thanks to the careful work of Ransom and Hall which has extended over several years it is now known that the larvae of *G. scutatum* occur in various species of dung beetles and that they have been raised experimentally not only in these but also in croton bugs. Furthermore, eggs of a *Gongylonema*, most probably *G. pulchrum* from the esophagus of a hog, were fed to croton bugs and at the close of a month embryos were found encysted in the final stage and ready for transmission.

In the present case it is easy to see how an infected insect, very likely a croton bug, might have been ingested whole, or some fragments of it included by accident in meal, flour, milk, or other materials used in cooking. Such sanitary mishaps are very common with poorly prepared food, and thus the infection of the human host would have been achieved. The sanitary conditions noted in the clinical history of the case favor such an occurrence.

The duration of the infection can be estimated as at least a month if the clinical symptoms stand in any connection with the parasite. No data have been found to show the rate of development of these worms in the final host but if one can infer safely from other nematodes it is likely that this specimen was in the human host more than a month and that the symptoms did not manifest themselves until it had attained a certain measure of growth. These symptoms were so definite and terminated so promptly with the removal of the parasite that etiologic significance can hardly be denied to the worm.

SUMMARY

A specimen of *Gongylonema*, probably *G. pulchrum*, has been recovered from man. This species is normally a parasite of the pig.

PLATE

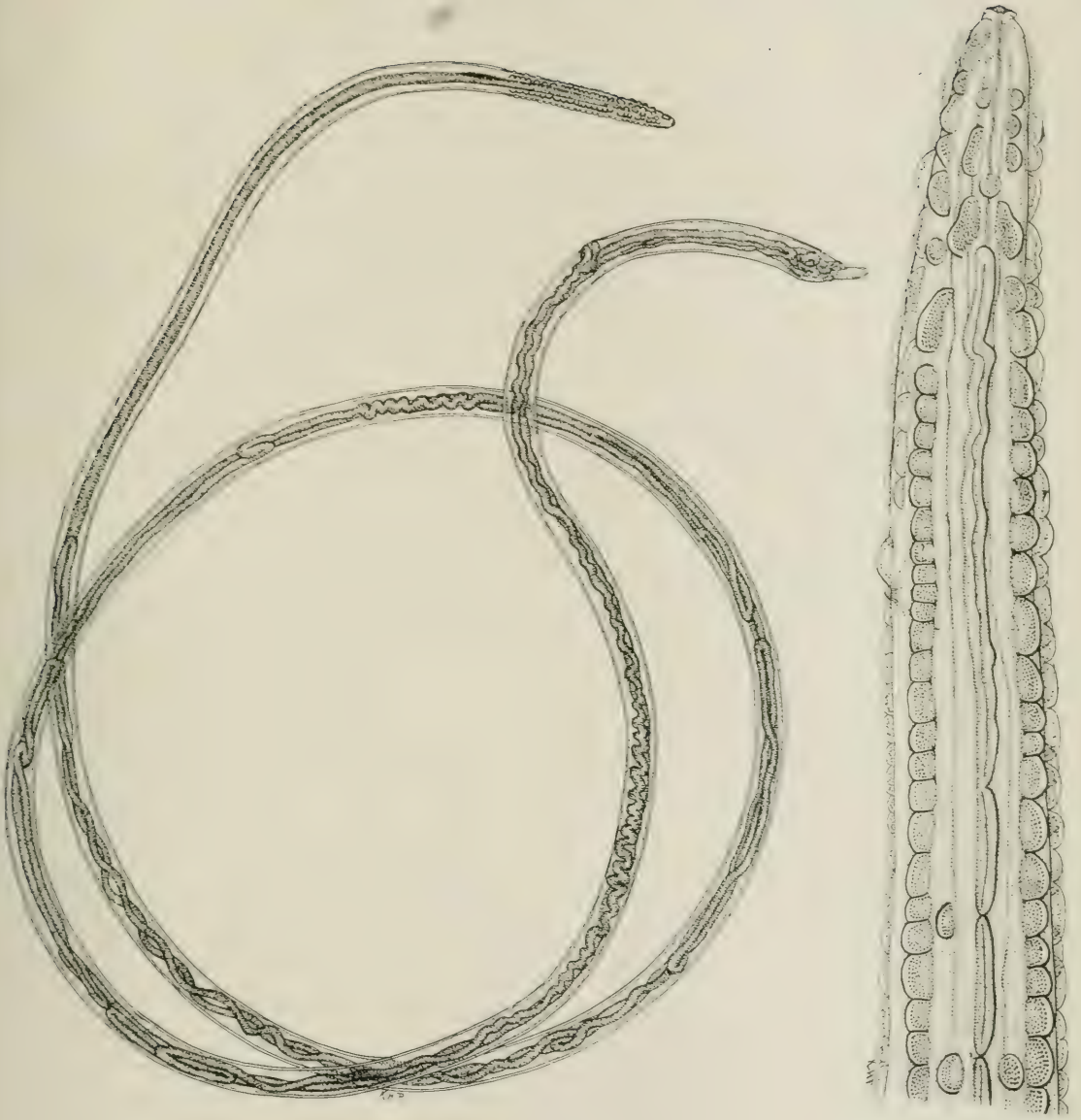


Fig. 1

Fig. 2

EXPLANATION OF FIGURES

Fig. 1.—*Gongylonema* (*pulchrum*?) from human host. Camera drawing. X 15.

Fig. 2.—Anterior end of same specimen. Camera drawing. X 140.

Infection of the human host was brought about probably by the ingestion of larvae in the infective stage which had developed in some insect. Very likely the croton bug, known by experiment to be able to serve as intermediate host for this species, was the source of the infection which might readily occur by accident.

The presence of the parasite was accompanied by clinical symptoms indicating marked digestive and nervous disturbances, associated with anemia. These symptoms disappeared with the removal of the worm.

The parasite displayed a tendency to wander through the sub-mucosal connective tissue from the lips to the throat.

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ARE SARCOSPORIDIA ABERRANT FORMS OF CNIDOSPORIDIA OF INVERTEBRATES?

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In the year 1910 v. Rätz stated that Sarcosporidia are very near to the Nosematidae. In an interesting paper Darling (1915) writes: "On account of the facility with which herbivora may obtain and ingest invertebrates infected with Neosporidia, but more particularly flowers, leaves, etc., or water contaminated with droppings of bees, moth larvae, fly droppings, etc., or other material containing Neosporidia spores, is it not possible that Sarcosporidia may be side-tracked varieties of some of the Neosporidia of invertebrates which have invaded the musculature of a hospitable though by no means definitive host and, being unable to continue further their life cycle, have escaped from a compromising and aberrant position?"

This opinion of Darling was supported by the experiments of Scott (1915) who concludes his paper with the following words: "All of the evidence favors the view that the sheep is not the definite host of *S. tenella* and, therefore, is in accord with Darling's suggestion that the muscle parasites of vertebrates are aberrant forms."

In such an order of ideas it is perhaps interesting to note observations which seem to me to support the hypothesis that Sarcosporidia are identical with Cnidosporidia.

In 1896 Piana, in a paper on the life cycle of *Balbiania gigantea*-*S. tenella*, states that in the cultures of this parasite on sterilized moist filter paper, moist earth, gelatine prepared with *Focus crispus* at a temperature of 18 to 25 C. in twenty-five to sixty days the spores give bodies which develop more and more and present a nucleus and amoeboid movements. The movements cease after some days and the amoeba assumes the form of a cyst. Piana tried to infect a sheep per os with this material, but with negative results. I may add the macroscopic examination made with a view to finding cysts of *Balbiania*, was confined to the esophagus.

After seventeen years I repeated (1913) the experiments of Piana with *S. muris*, making cultures of this on sterilized filter paper moistened with physiological salt solution at a temperature of 20 C. After eight days I found in this culture amoeboid bodies showing slow movement at a temperature of 20 and 37 C. This amoeba had a fine granulated protoplasm, a nucleus, and clear vacuoles. Colored with Leishman or Giemsa the protoplasm assumed an azure color, and the

nucleus a red color. After thirty-two days the cultures presented only encysted forms. With this culture I inoculated a white rat and a black mouse in the muscles of the thigh but without result. The inoculation of a white rat with *S. muris* was negative, as was also the infection per os of a guinea-pig with the same *Sarcocystis*. Neither Piana nor I affirm that the amoebae were positively derived from the spores of *Sarcocystis*, but we stated that it was very probable; and the observation of Erdmann (1914) that in the intestinal cells of a mouse infected per os with *Sarcosporidia* appear little amoeboid bodies, seems to support our experiments and the fact that *Sarcosporidia* are identical with *Cnidosporidia*. This idea was also expressed by Auerbach (1910) who says: "Mit der Tatsache des vorhandenseins einer Polkapsel bei *Sarcosporidiensporen* fällt ein wesentlicher Unterschied gegenüber den *Cnidosporidien* fort und es liegt kein zwingender Grund vor, sie noch von diesen zu trennen."

If it is possible to demonstrate that spores of *Sarcosporidia* produce amoebae, the identity of the two orders is more probable. The drawings of the amoebae of *Myxidium inflatum*, *leptotheca macrospora* and *Myxidium bergense* in Auerbach (1910: 10, 79) are very likely the drawings of amoebae of *S. muris* shown in my paper.

Given the spores of the *Sarcosporidial* amoebae, the infection with *Sarcosporidia* must be analogous with the infection with *Cnidosporidia*. The experiments of Thélohan and particularly those of Auerbach on the development of the *Cnidosporidia* in the intestine of fishes prove that the spores of this parasite produces amoeboid bodies in the duodenum. It is probable that the spores of *Sarcosporidia* must also produce amoebae in the intestines and perhaps in Piana's experiments the amoebae were destroyed in the stomach. In fact Auerbach failed to observe a transformation of spores of *Cnidosporidia* in the stomach of fishes. I think it would prove an interesting experiment to make cultures from the great cysts of *S. tenella*, and to introduce the amoebae directly into the intestines of sheep. With such a method of infection it is perhaps possible to secure much more positive results than to work with spores of *Sarcosporidia* which as Auerbach states are in some cases probably not ripe.

SUMMARY

1. The observations of Piana and Galli-Valerio to the effect that spores of *Sarcosporidia* produce amoebic bodies in cultures, more closely relate the *Sarcosporidia* to the *Cnidosporidia*.
2. If true that *Sarcosporidia* are only aberrant forms of *Neosporidia* of invertebrates, then the hypothesis of Darling becomes more probable.

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THREE NEW GREGARINES FROM MARINE CRUSTACEA *

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The protozoan parasites described here were studied during the summers of 1913 and 1914 at the Biological Laboratory of the Brooklyn Institute, Cold Spring Harbor, L. I., and I wish to thank Dr. C. B. Davenport, the director, for kindly affording me the privileges of the laboratory.

All the hosts were found on the Sand Spit, a narrow peninsula half a mile long separating the outer and inner harbors. The area is thus geographically very restricted, and although the hosts are similar and the possibility of all of them being infected with the same parasite considered, yet there is abundant evidence to warrant regarding the three forms described below as separate species.

Frenzelina delphinia nov. spec.

Figures 1 to 8

The host of this gregarine is the large white sand flea, *Talorchestia longicornis*, which is found in fine sand between tide marks. Fleas were taken from the eastern end of the Sand Spit, and also from locations along the road to Lloyd's Neck, from Huntington Beach, and from Northport Harbor; and hosts from all these localities were found to be infected.

The parasites were present in 30 per cent. of the 260 intestines examined, the number in an infection varying from one to ten in 75 per cent. of the hosts and from ten to 500 in the other 25 per cent.

The sporonts (Figs. 1 and 2¹), which live free in the lumen of the upper part of the intestines, are small, the average of fifty large individuals being 110μ in length and 60μ in width. The largest individual seen measured 115μ in length and 64μ in width. The ratio of length of protomerite to total length of sporont is about 1:4; the ratio of width of protomerite to width of deutomerite about 1:1.5. A table of a few measurements is given at the end of this section.

The sporonts are biassociative when mature and the two members of an association do not differ materially in size; either the primitive or the satellite may be a little longer or broader than the other. The sporonts are stout bodied, being less than twice as long as wide. The

* Contributions from the Zoological Laboratory of the University of Illinois, under the direction of Henry B. Ward, No. 60. Also from the Biological Laboratory, Cold Spring Harbor.

¹For explanation of special terms used see description of Figure 1.

protomerite is cylindrical and broadly rounded in front and is about three fourths as high as wide. There is often a slight constriction at the septum which separates the protomerite and deutomerite. The deutomerite is doliform, two thirds as wide as long, widest through the middle portion, and terminates in a broadly rounded extremity. There is a small conspicuous papilla at the anterior end of the protomerite of the young sporonts which persists, although reduced in size, in the adults. In the satellite of an association it becomes a structure for the attachment of the two sporonts by projecting upward and forming a small indentation in the posterior end of the primate (Fig. 4). Hydrochloric acid, either dilute or concentrated, fails to dislodge the two sporonts, but sodium hydroxid or ammonium hydroxid, in solution, readily disassociates them.

The endocyte of the mature sporonts is light brown in color and in full-grown animals fairly dense, and the nucleus not visible. Young sporonts are less dense and the color paler. The satellite of an association is generally less dense than the primate, and for this reason its nucleus is often visible in vivo when that of the dense primate is not. The protomerite of a sporont is slightly less dense than the deutomerite and contains fewer protoplasmic granules; the granules are slightly larger than those in the deutomerite and the endocyte paler in color. When the host has just been taken and has probably fed the night before, full grown sporonts are dense; when, however, the fleas have been kept in damp sand for a few days without food, the parasites are likewise deprived of nourishment and become pale in color and the number of protoplasmic granules present, hence the density, is greatly reduced. The addition of a drop of iodine solution renders these pale parasites visible but of course kills them.

The nucleus of the sporonts is generally visible in vivo only as a lighter indefinite area although in young and starved sporonts it is often visible. It is large and spherical and contains one or two large homogeneous karyosomes. The epicyte is thin and fragile and is marked with longitudinal striations only visible under an oil immersion lens.

Cross sections made of the intestine of the host reveal the fact that development is intercellular as in the family of the Stenophoridae. This is the first genus of the family Gregarinidae which has been found to develop in this manner, the other members possessing an epimerite which alone is attached to the host cell, the remainder of the trophozoite projecting into the lumen of the intestine. In the species here described, the whole trophozoite is embedded in the epithelium (Figs. 3 and 6). When stained with Delafield's hematoxylin, the cells of the epithelium become purplish-blue, while the parasites stain less deeply and the color is a clear homogeneous blue with a darker blue nucleus.

The animals are capable of movements both of bending and of gliding progression. The sporonts are able to move through a narrow place in the manner employed by an amoeba. When the host intestine is first opened, a mosaic of inert distorted individuals or associations lying near the epithelium is often revealed; when, however, water or normal salt solution is added, the smooth, regular contour is quickly restored. Normal salt solution stimulates movement and sporonts often remain alive and motile for an hour and a half. Sea water has the same effect. As motion tends to be retarded at the end of this time, weak tannic acid solution was added in a few instances and caused considerable acceleration of movement; plasmolysis, however, occurred inside of another half hour and the animals died.

That transparent threads of mucus are present at the posterior end of the body was frequently attested by the fact that the animals were able to carry with them in their movements large or small masses of debris at a distance behind the body often as great as the length of the animal itself. A mass twenty-five times the volume of the gregarine itself was in one instance observed being carried along.

Cyst formation was observed in material from the host intestine from its incipency until rotation ceased. The adult sporonts free in the intestine which are ready for cyst formation become thickened and shortened, motion becomes sluggish, movement of progression ceases and that of bending becomes more active (Fig. 5). Concomitant with the revolving motion there occurs a deposition of gelatinous threads exuded from the body in fine concentric layers around the revolving mass. The sporonts become a spherical mass and the threads form a thick cyst wall. The rotating mass passes from the mid intestine to the rectum and ceases motion (Fig. 7). It begins here its development by the loss of the wall separating the two sporonts and the disintegration of the two sporont nuclei. The protoplasm of the cyst collects in masses and on the periphery of each protoplasmic mass are formed spherical protuberances, the gametids. The cyst is now expelled with the feces. Cysts when expelled are somewhat opaque, tan in color, and average 80μ in diameter, including the cyst wall.

It is difficult to effect cyst development to completion by artificial methods. Marine bacteria seem to be virulent and to cause putrefaction or otherwise stop development in the early stages. Cysts were kept in the damp chamber in normal salt solution and a few yielded gametes upon being crushed when 24 hours old. The gametes were stained with safranin; they were large and nearly spherical, and no difference in size was observed in those from the same cyst. Some of the gametes, however, showed large deeply staining nuclei and others smaller nuclei which stained less deeply, probably because of their reduced chromatin

content. A difference in the staining reaction was also noted in the two sporonts in a cyst of about eighteen hours, the one sporont mass staining more deeply than the other.

A very few cysts developed to completion and dehiscid by simple rupture in 35 hours, but no well formed spores were present.

Orchestia agilis, the smaller very common sand flea, was found in one instance to be infected with three nonassociative sporonts which agree in size and shape with the species described above. This flea was also heavily parasitized with an infusorian. *Orchestia grillus* from the roots of the eel grass (*Juncus palustris*) was not found to be infected with gregarines.

The genus *Frenzelina* (Léger and Duboscq, 1909) has not hitherto been reported from the United States and only seven species have been described. The species described above is placed in this genus because (1) the sporonts are biassociative, (2) the cysts dehiscid by simple rupture, (3) development is intercellular, (4) the apex of the protomerite is slightly papillated, (5) the parasite inhabits the intestine of a crustacean.

I wish to designate the species, of which no previous record is found, *Frenzelina delphinia*.

The fact that development is intercellular was not determined by Léger and Duboscq and this important fact should be added to the features which characterize the genus *Frenzelina*.

A table of typical measurements, in microns, follows:

Total length association.....	215	215	210
Primate:			
Total length sporont.....	115	110	112
Length protomerite	20	24	27
Length deutomerite	95	86	93
Width protomerite	39	35	43
Width deutomerite	62	66	63
Ratio length protomerite: length deutomerite	1:4.7	1:4.6	1:3.5
Ratio width protomerite: width deutomerite	1:1.6	1:1.8	1:1.5
Satellite:			
Total length sporont.....	100	105	98
Length protomerite	20	20	20
Length deutomerite	80	85	78
Width protomerite	37	40	40
Width deutomerite	55	56	60
Ratio length protomerite: length deutomerite	1:1.5	1:1.4	1:1.5
Ratio width protomerite: width deutomerite	1:1.5	1:1.4	1:1.5
Cyst, outer diameter.....	77	90	86
Inner diameter	63	74	66
Thickness transparent layer around cyst..	7	8	10

Frenzelina olivia nov. spec.

Figures 8 to 10

The host of this species is the small littoral spider crab, *Libinia dubia*, which is abundant along the shores of Long Island Sound and its inlets. The parasite is found in the upper part of the intestine. It generally occurs in moderate numbers (10 to 100) but rarely an infection of 1,000 or more is encountered.

The sporonts are biassociative and average 80μ in length and 35μ in width. They are ellipsoidal in shape, rounded in front and rather blunt posteriorly. The protomerite is hemispherical, very slightly constricted at the septum. It is about one fifth the total length of the sporont and slightly papillate in the adults (Fig. 8); the younger solitary sporonts possess a conspicuous papilla (Fig. 10). The deutomerite is but little wider than the protomerite (1:1.2); in solitary individuals it is more broadly rounded at the posterior end than in those which are attached. The endocyte of the mature sporonts is dark brown and very dense in the deutomerite; the protomerite is less dense and tan in color. At the anterior end of the protomerite is an orange colored disc. The nucleus is visible only in immature specimens. It is spherical and generally contains one large karyosome.

Movement of progression is rapid and continues only for intervals of about two seconds.

Cysts are spherical, dark brown in color, and from 45 to 60μ in diameter including the enveloping wall. They occur in the posterior third of the intestine. No sections were made of the host intestine.

This species is placed in the genus *Frenzelina* because (1) it is biassociative, (2) there is a papillated conspicuously differentiated apical area in the protomerite, (3) it is very similar to *Frenzelina delphinia* in form and location and both occur in hosts from the same habitat; (4) it is parasitic in the intestine of a marine crustacean.

The larger spider crab, *Libinia emarginata*, which is found in deeper water and seldom comes near the shore, has been examined repeatedly for gregarines, but none have been found to date. Other crabs procured from oyster boats, and which were dredged in the Sound and Harbor, have not yielded gregarines. These include *Neopanope texana sayi*, *Carcinides macnas*, *Pagurus bernhardus* and *Pagurus longicarpus*; and *Chloridella empusa* from the mud flats of the Inner Harbor. From the south side of the island, the following crabs have been examined and none found to be infected with gregarines: *Emerita talpoida*, *Callinectes sapidus*, *Ovalipes ocellatus*, *Ocypoda albicans*, and an undetermined species of *Orchestia*.

It seems possible that only littoral marine crustacea are infested with gregarines and that spores are eaten along with shore vegetation,

grasses and tide water algae, and are swept away by the tides or are noninfective when they reach the water.

A table of measurements, in microns, follows:

Total length association.....	218	195	150	127
Primitive:				
Length sporont	100	85	80	65
Length protomerite	20	20	14	14
Length deutomerite	80	65	66	51
Width protomerite	35	38	30	30
Width deutomerite	43	48	45	36
Ratio length protomerite: total length sporont	1:5	1:4.2	1:5.7	1:4.6
Ratio width protomerite: width deutomerite	1:1.2	1:1.3	1:1.5	1:1.2
Satellite:				
Length sporont	118	110	70	62
Length protomerite	25	14	10	10
Length deutomerite	83	96	60	52
Width protomerite	36	39	22	22
Width deutomerite	36	50	28	30
Ratio length protomerite: total length sporont	1:5	1:8	1:7	1:6.2
Ratio width protomerite: width deutomerite	1:1	1:1.3	1:1.3	1:1.4

Frenzelina nigrofusca nov. spec.

Figures 11 to 14

Two species of fiddler crabs, *Uca pugnax* and *U. pugilator*, which live together at the roots of the eel grass, were found to be infected with a species of gregarine. About 30 per cent. of the hundred or more crabs examined were parasitized and the infection was very moderate; in only rare instances was the number of parasites present greater than fifteen.

The sporonts were solitary, none being associative as is characteristic of this genus. The body is broadly ovoidal, less than twice as long as wide (Fig. 11) and is often nearly rectangular in shape with rounded corners (Fig. 12). Sporonts average 100μ in length and 65μ in width. The protomerite is hemispherical and very slightly constricted at the septum; it is about one third the length of the whole sporont. There is a minute papilla at the anterior end; this papilla is large and conspicuous in the trophozoite (Fig. 13). The deutomerite is of approximately the same width as the protomerite and twice as long. It is very broadly rounded or often flattened posteriorly.

The endocyte is very dense and appears dark brown or black in transmitted light. It is but little less dense in the protomerite than in the deutomerite and in starved animals becomes tan in color. The nucleus is not visible in the live sporonts. The sarcocyte is relatively thick, especially over the anterior end of the protomerite. The nucleus

is small and spherical and contains one or two minute karyosomes. Uniform gliding movement was observed at a relatively slow rate. The cysts are very dense and are spherical. Spores were not seen.

This species is placed in the genus *Frenzelina* for two reasons: (1) the protomerite possesses at its apex a small papillated and conspicuously colored disk, this papilla being well developed in the trophozoite, (2) the gregarine infects the intestine of a marine crustacean. While no associative sporonts were seen, the species is very probably associative from its affinities.

A table of measurements of a few specimens is appended here, all dimensions being given in microns:

Total length sporont.....	72	82	100	120	125
Length protomerite	20	22	22	31	31
Length deutomerite	52	60	78	89	94
Width protomerite	39	40	70	50	75
Width deutomerite	37	40	75	75	65
Ratio length protomerite: total length	1:3.6	1:3.9	1:4.5	1:4	1:4
Ratio width protomerite: width deutomerite	1:1	1:1	1:1	1:1.5	1:2.1
Diameter nucleus	1	1.1	1.5		

SUMMARY

1. Three new Gregarine parasites of the genus *Frenzelina* are described from marine Crustacea.
2. Parasites belonging to this genus have not hitherto been reported from the United States.
3. A new definitive character for this genus has been determined upon sectioning the host intestine, namely, the fact that the parasites are intercellular.

REFERENCE CITED

- Léger, L., and Duboscq, O. 1909. Etudes sur la sexualité chez les Grégarines. Arch. Prot., 17: 19-134.

EXPLANATION OF PLATE

Frenzelina delphinia nov. spec.

1. An association of two sporonts from lumen of intestine of *Talorchestia longicornis*; *a*, protomerite of sporont, *b*, deutomerite, *c*, primite, *d*, satellite.
2. Another association with satellite much younger than primite.
3. Cross section of portion of intestine of *Talorchestia longicornis* showing intercellular development of parasite; *a*, oblique section of trophozoite embedded in epithelium; *b*, *c*, sections of sporonts lying free in lumen but near the walls and surrounded by mucus.
4. Interlocking device by which satellite of association is attached to primite.
5. Association revolving in an early stage of cyst formation.
6. Cross section of portion of host intestinal epithelium showing embedded trophozoite in longitudinal section.
7. Cyst soon after completion, showing transparent cyst wall and two sporonts still distinctly outlined.

Frenzelina olivia nov. spec.

8. Mature association from lumen of intestine of *Libinia dubia*.
9. Immature sporont from same location.
10. Young sporont free in intestinal lumen, showing papilla at anterior end of protomerite. Magnification greater than in Figures 8 and 9.

Frenzelina nigrofusca nov. spec.

- 11, 12. Adult sporonts from lumen of intestine of *Uca pugnax*.
13. Trophozoite from lumen of host, showing papilla.
14. Sporonts with posterior half of body contracted, indicating that considerable movement of the body is possible.

PLATE



THE PAJAROELLO TICK (*ORNITHODORUS CORIACEUS* KOCH)

WITH SPECIAL REFERENCE TO LIFE HISTORY AND BITING HABITS

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For several years previous to beginning his observations on this species, the writer has listened to many harrowing tales about the *Pajaroello*. No one seemed to know exactly what it was and no one seemed to have collected specimens so as to make accurate identification possible in so far as the writer knew at the time. Complaints came almost exclusively from the more mountainous portions of Santa Clara and San Benito Counties (California). Natives, principally Mexicans, in the vicinity of Mt. Hamilton fear this parasite more than they do the rattlesnake, and tell weird tales of this or that man having lost an arm or leg, and in one instance even death having ensued, as the result of a bite by the *Pajaroello*. There seems to be a superstition in that region that three bites will result in certain death. The stories all agree in the essential detail that the bite results in an irritating lesion which is slow to heal and often leaves an ugly deep scar. Several persons also informed the writer that the *Pajaroello* occurred in certain mountainous portions of Mexico. It was not, however, until August, 1913, that living specimens came to hand, taken in Santa Clara County in the vicinity of Mt. Hamilton. These were identified as *Ornithodoros coriaceus* Koch, described in 1844 from a single female specimen from Mexico. A translation by Nuttall of the original description is as follows:

"Shaped like the sole of a shoe, thick margined, roughly shagreened, yellowish earthy color, spotted rusty red, legs toothed dorsally. Length 9.3 mm. Body about twice as long as wide, width fairly uniform, indented on the sides, pointed above the mouthparts, rounded posteriorly, a thick turned-up border all around; the whole surface above and below thickly granulated like fish skin (shagreen), the granules flat above, consequently, the whole leathery, on the back unequal folds and grooves. Beneath in the front of the body a deep groove running to the stigmata and on the inner protrusion the rather large round quite clearly marked eyes. The coxae gradually thicken toward the distal extremity and are somewhat bent; the other articles somewhat compressed and clearly notched or round-toothed. The whole surface, above and below, dirty yellowish earthy color, rusty red spots irregularly distributed throughout. Capitulum and palps light yellow. Legs gray-brown. Female. Male: unknown. Habitat: Mexico."

From either specimens received or reliable information at hand it now seems evident that this species occurs in the more mountainous portions of the following counties in California, namely, San Benito, Santa Clara, Stanislaus, Monterey and Santa Barbara, probably also Los Angeles and San Diego, thus connecting up with Mexico, which is probably the original habitat. The tick is most commonly found among the dry leaves beneath live oak trees where cattle are accustomed to lie in the shade. Most cases of tick bite caused by this species have occurred while sitting or lying down in such situations.

This species of tick is a typical representative of the genus *Ornithodoros* of the family Argasidae and is superficially not greatly unlike the relapsing fever tick of Africa, namely, *Ornithodoros moubata* Murray.

Since August, 1913, the complete life history of this tick has been worked out and much information has been gained relative to its habits and venomous properties. In this work the writer has been greatly assisted by several of his advanced students, notably Mr. W. L. Chandler, who has undertaken an exhaustive study of this species.

LIFE HISTORY

Six adults and half-grown specimens, males and females, were secured during the month of August, 1913. Of these Tick No. 6, a fully grown female, engorged on blood from a Rhesus monkey, November, 1913, deposited a lot of eggs Feb. 13, 1914, and continued to lay eggs at intervals during the rest of that season. Various experiments were performed with the ova and the several larvae resulting from the first laying, mainly for the purpose of determining the best method of procedure. From the second laying of this tick we secured our first complete life history data as follows: March 9, Tick No. 6 deposited 323 ova, hatched March 31; giving an incubation period of about 21 days at an incubator temperature averaging 26.3 C. (variation ± 1 C.). The larvae were placed on the ear of a rabbit May 2 and among others one was recovered fully engorged May 11, and given the number 18. The first moult occurred May 21, giving about 51 days for the larval stage in this instance. The second moult without a second engorgement took place June 15. The nymph became fully engorged in about twenty minutes on July 2, the third moult occurring August 12. Becoming fully engorged again October 11, the fourth moult took place December 23. Engorging again Jan. 16, 1915, the fifth moult took place March 9 and the sexually differentiated tick (a female) appeared. March 27 it became fully engorged on a mouse and was placed with male No. 3 on April 16, copulation taking place April 17. The first laying consisting of 428 eggs took place June 10, 1915. Thus the egg to egg period in this

individual covered exactly fifteen months. This time can be reduced very considerably by applying the ticks to a suitable host animal at shorter intervals, indeed we have one record of a male in which sexual differentiation was accomplished in 159 days, as against 343 days in Tick No. 18, a female. Under natural conditions it seems quite probable that there is one generation each year and that two years may be necessary in many instances.

Although the incubation period at a given sustained temperature suffers little variation, e.g. at 26 C. it is 21 days, the length of time required for the other stages varies considerably, depending on the presence of a host mainly.

The minimum length of the larval period was found to be 19 days. The number of moults varies from four to seven.

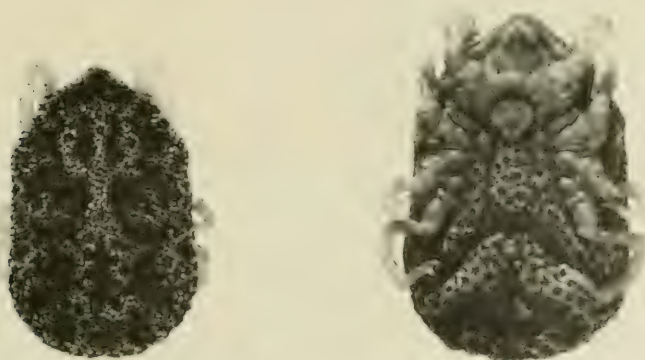


Fig. 1.—The Pajaroello tick, *Ornithodoros coriaceus* Koch. Dorsal, left; ventral, right.

The length of time a female may remain fertile without further copulation is at least two years as shown by the fact that Tick No. 6 received as a fully grown individual August, 1913, and not thereafter placed with a male, deposited eggs during the summer of 1914 and 1915. The total number of eggs deposited in one season by Tick No. 6 was 1,158, there being seven separate layings. The maximum number of eggs deposited in a single lot for the year 1914 by Tick No. 6 was 323. This same tick, however, deposited 802 eggs April 26, 1915, her tenth laying in captivity, and her daughter, Tick No. 18, deposited 428 in her first laying.

We have experienced no little difficulty in rearing this species of tick. However, the ear of a rabbit is best suited for feeding the larval ticks; later stages are best fed by placing the ticks either on a rabbit or on a mouse, holding these with the hands until the ticks have become fully engorged and drop off, this process requiring from 15 to 30 minutes.

BITING HABITS AND VENOMOUS NATURE

Mr. W. L. Chandler, a graduate student in the University of California, formerly with the United States Public Health Service, has given the writer an accurate account of two bites which he suffered while stationed in the San Antone Valley (California). The first bite was received July 2, 1912. He experienced a sharp pain on the left arm and upon rolling up his sleeve discovered a large tick, partly engorged, attached to the upper arm in front. He dislodged the tick and sucked the lesion. The lesion when first discovered showed a small dark purple ring surrounding a bright red spot, the point of attachment. The discoloration disappeared in a short time but the arm was "highly irritable for two or three days and at the point of attachment a minute clear scab formed." The tick proved to be a pajaroello.

The second bite took place July 16 while seated in a thicket of willows (the first bite took place while riding over a brush grown hill), and in this case the sharp pain involved the left leg. An almost fully engorged tick, again a pajaroello, measuring about three-fourths of an inch in length and about one-half inch in width was removed from just above the shin. Once more a bright red spot was visible at the point of attachment surrounded by an irregular purple ring about three-fourths of an inch in diameter. In about half an hour the leg began to swell in the vicinity of the lesion and in about three hours the entire lower leg was tremendously swollen. The coloration about the point of attachment had widened considerably, was puffy and a clear lymph exuded from the lesion. The young man lanced the leg causing the blood to flow freely and treated the wound with crystals of potassium permanganate, binding the leg with cotton and gauze. During the following night he reports experiencing a general disagreeable feeling, the entire lower leg being "irritable and numb." On the following day the bite on the arm became irritable again, and was treated as had been the leg, fearing bad results. For several weeks both lesions exuded a clear lymph from beneath an "oily looking, transparent, red mottled scab, which remained in evidence for two or three months."

Chandler reported these ticks very numerous in some localities, having counted as high as six within half an hour crawling over a saddle blanket placed on the ground. Their presence and number seemed to be determined by the presence of cattle, although ticks were found where there were no cattle but in places which were evidently favorite haunts of wild animals.

EXPERIMENTS WITH THE PAJAROELLO

On monkeys: A number of specimens of *Ornithodoros coriaceus* were collected in the San Antone Valley and at Newman, California, for purposes of experimentation and study of life history. In coopera-

tion with Dr. W. A. Sawyer and Messrs. S. W. Newman and W. L. Chandler, the writer conducted a number of experiments particularly with reference to the bite. In one of these experiments a mature female tick was permitted to bite a nearly full grown monkey (*Macacus rhesus*) twice within an interval of sixteen days intervening between the two bites. The tick was applied at 9:42 a. m. Dec. 10, 1913, and began sucking blood at 9:43, one minute later, becoming engorged and falling off at 10:21 a. m., a period of 38 minutes. At 10:30, a few minutes after the tick dropped off, there appeared a deep red hemorrhagic area 2 mm. in diameter at the point of biting with a somewhat lighter area 10 mm. in diameter surrounding the central area. At 10:47 there was a black spot at the point of bite 1.5 mm. in diameter. the inner red hemorrhagic area measuring 4 mm., with a yellowish white area surrounding this 8 by 6 mm., and an outer petechial area 15 by 13 mm. No general symptoms were noted. The lesion reached its greatest expanse the following day when the following measurements were taken: dark purple spot 2 mm. in diameter (a very dark red scab); the inner red area 6 by 5 mm., the yellowish white area 20 by 12 mm., the outer area 48 by 23 mm. and fading. The yellowish white area including bite was slightly swollen. By December 14, i.e. four days after the bite was received, the ecchymosis had entirely disappeared; by December 16, six days after the bite, the lesion was entirely gone but for a slight pigmentation, a thickened reddish area measuring 5 by 3 mm. and a small scab 2 mm. in diameter.

The monkey remained normal throughout the experiment as regards temperature, weight, blood count and general condition.

The second bite was received by the same animal on December 26, the tick being applied at 9:43 a.m., taking hold at 9:44 a.m. and dropping off fully engorged at 9:55 a.m., requiring but 11 minutes to engorge. The history of the second bite follows that of the first very closely, except for the extent of the lesion which was greater, i.e. 70 by 30 mm. In order to note any manifestation on the part of the first lesion, the second bite was located near the opposite nipple. No change was observed. The lesion produced by the second bite had disappeared by December 31, i.e. five days after the bite, except for a slight thickening 3 mm. in diameter and a slight white scale at the center. Again the monkey had remained normal, except for a slight increase in the count of white blood corpuscles which rose from 7,400 at the time of the bite to 13,900 by noon of the same day, going down again to 7,300 by 5 p.m.

Both nymphs and adults readily attach to man, monkey, rabbit and mouse; and become fully engorged in from 15 to 30 minutes, depending on the number and length of rests in the rhythmic motion of the basis capituli. If dislodged while engorging, they, like the larvae, refuse to reattach immediately.

On rabbits: The bite on a rabbit's ear leaves a comparatively large, thick, purple nodule, and is accompanied by a more or less hemorrhagic condition of the entire ear tissue. The bite has no apparent systemic effect on the rabbit, and the lesion heals within two or three weeks.

On mouse: When a tick was applied to the body of a mouse for the first time, the mouse showed no apparent uneasiness until the tick attempted to withdraw its head. Beginning immediately after the tick dropped from the mouse there occurred a small swelling which exuded lymph, rapidly grew in proportions and was accompanied by marked ecchymosis. In 2 minutes after the tick had dislodged itself the swelling had increased its area 0.3 by 1 cm., in 30 minutes 0.5 by 2 cm., and in one hour 0.5 by 2.5 cm. After 24 hours the swelling had become reduced, except in the vicinity of the bite, and fully one half of the mouse was dark blue. But slight systemic disturbances were noticeable in the mouse, and it rapidly recovered. The same mouse was used as a host for other ticks, and each succeeding bite produced less and less noticeable results, until finally only very slight lesions were produced.

SUMMARY

The venomous Pajaroello Tick ("Pajahuello" according to Banks) is a native of Mexico, but is now known to occur in the more mountainous coastal counties of California as far north as Santa Clara within 100 miles of San Francisco.

The breeding habits, metamorphosis and life history have been carefully observed in the Parasitology Laboratory of the University of California. . Records of life history in individual cases (i. e., from egg to sexual maturity) show 159 days for the male and 343 days for the female at a temperature averaging 26.3 C. (variation ± 1 C.). The venomous nature of the bite as affecting man, monkey, rabbit, and mouse is described.

NOTE ON THE ETIOLOGY OF VERRUGA AS DEDUCED
FROM A STUDY OF THE ASEQUAL STAGES
OF BARTONELLA

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Dec. 4, 1915, the writer presented before the Biological Society of Washington a paper on the identification of the asexual stages of *Bartonella bacilliformis*, the causative organism of verruga, which was published in the Dec. 19, 1915, issue of the *Journal of the Washington Academy of Sciences*.

Jan. 7, 1916, he presented the same subject before Section VIII of the Second Pan-American Scientific Congress, during which presentation he announced certain points additional to those previously announced. This second paper will be published in due course in the Proceedings of the Congress, but it is desirable to place on record at once the additional points announced therein. They are as follows, and should be added to the paper published Dec. 19, 1915:

The toxin resulting from the extensive asexual multiplication of *Bartonella* in the vascular endothelial cells of the subcutaneous tissues is liberated in quantity into the blood, causing the rise of temperature which marks the fever stage of verruga, the anemia following through hemolysis.

The proliferation of vascular endothelial cells incited by this toxin not only imprisons the toxin itself, thus arresting the hemolysis, but also prevents the erythrocytes from coming into direct contact with endothelial cells containing merozoites of *Bartonella*, thus cutting short the infection of the erythrocytes. As the natural result, the fever and anemia both subside, and the gametes of *Bartonella* are no longer to be found in the peripheral blood.

The infected endothelial cell, in situ in the capillary wall, is positively chemotropic for uninfected freshly oxygenated erythrocytes, attracting and holding them in contact with itself until transfer of a certain number of merozoites of *Bartonella* has been effected, the presence of which reduces the oxygen tension in the substance of the erythrocytes, thereby transforming their tropic qualities, the sufficiently infected erythrocytes being set free through negative chemotropism.

The localized proliferation of vascular cells following verruga eruption-tissue inoculations is not due to any new activity of a living organism or virus. The reason why Drs. Strong *et al.* were unable to obtain proliferation lesions by injection of a filtrate from these tis-

sues is at once apparent; the proliferated vascular cells can not pass the filter. Their inoculation of these tissues upon the rabbit's cornea produced no lesion because the cornea possesses no vascular cells. Their attempts to cultivate the supposed virus in these tissues resulted in failure because the tissues evidently do not contain a living virus. (The term virus is used in the common acceptation of organisms that pass the filter.)

Drs. Strong *et al.* succeeded in demonstrating the presence in verruga eruption tissues of a hemolysin which is active in relatively high dilutions, and whose discovery is very much to the point in this particular connection. This hemolysin is quite certainly the toxic by-product of the reproductive activity of Bartonella in the subcutaneous tissues. It is the specific cause of the anemia of the fever stage of verruga. Further, it is the agent which directly incites the proliferation of the vascular cells, thereby causing the eruption lesions. In other words, this toxin is able to destroy such delicate structures as the erythrocytes; is able to irritate the more resistant vascular cells sufficiently to cause them to proliferate; but is unable to produce any effect on such highly resistant structures as the connective-tissue cells composing the cornea. Its proliferative action on vascular cells continues through many successive series of inoculations, but finally becomes attenuated and no longer effective.

In verruga cases, when the correspondence in intensity of fever and visible eruption is not well marked, it is practically certain that infection of the internal organs has become proportionately greater, resulting in an increased internal eruption.

Notwithstanding all criticisms that may be put forward, the writer is content to rest his thesis upon the evidence presented, and invites a comparison of the published figures and descriptions of the vascular cell inclusions of the fever and eruptive stages of verruga. He has verified the findings by similar inclusions in his own verruga sections and smears. There are many additional details apparent at the present writing which have not yet been entered into, but they are unnecessary to the main demonstration; it is only necessary to say that all the details fit in perfectly with the identification that has been given of the asexual stages of Bartonella, thus clinching the interpretation of these as presented by the writer.

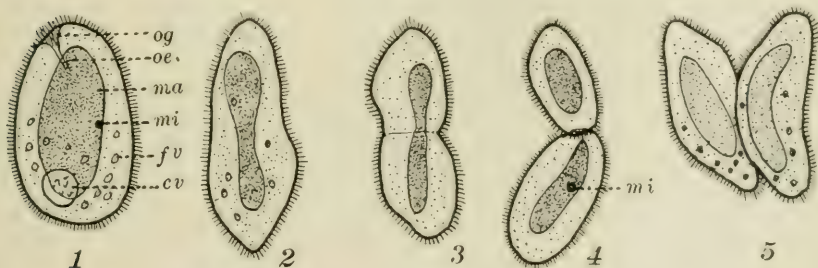
A NEW INFUSORIAN PARASITE IN SAND FLEAS*

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While examining some sand fleas on the shores of Long Island Sound for gregarine parasites, the writer found two hosts infected with an infusorian parasite; one of the hosts being the common small flea, *Orchestia agilis*, the other the larger *Talorchestia longicornis*. The two infected hosts were found in the same habitat and on the same day. The infusoria were found in only these two instances out of about 300 fleas examined for gregarines. The parasites infect the alimentary tract of the host in great numbers, several hundred being present in each flea.

In the vegetative stage, the animals are broadly ovoidal, tapering slightly at one end (Fig. 1). The macronucleus is very large, and ellipsoidal in shape; it is granular and homogeneous and does not



EXPLANATION OF FIGURE

1. *Balantidium orchestium*, vegetative individual. Og, oral groove; oe, esophagus; ma, macronucleus; mi, micronucleus; fv, food vacuole; cv, contractile vacuole.

2, 3, 4. Three stages in binary fission.

5. Conjugation.

stain deeply with Ehrlich's hematoxylin and acetocarmin. The micronucleus is small and deep staining, and lies contiguous to the macronucleus. The apical or subapical oral groove is small and inconspicuous; it leads to a short slender esophagus. Small contractile vacuoles were observed, not more than one being seen in a single individual. Many small food vacuoles were often present. Cilia of uniform length

* Contributions from the Zoological Laboratory of the University of Illinois, under the direction of Henry B. Ward, No. 61. Also from the Biological Laboratory, Cold Spring Harbor.

cover the body, those in the oral groove being slightly the longer. The size of vegetative forms ranges from 300 to 360 microns by 180 to 220 microns.

In individuals which are ready for asexual reproduction, the body becomes elongate and tapers at the ends; the macronucleus becomes longer and constricted centrally, at the same time contracting in volume. It also stains more deeply than does that of the vegetative animal (Figs. 2, 3, and 4). When transverse fission is complete, the two nuclei attain their normal vegetative form and density. Conjugation was noted in one instance.

The parasite may be classified as follows:

1. Order Heterotricha: zone of large cilia leading to mouth.
2. Suborder Polytricha: body covered with an even coat of cilia.
3. Family Bursaridae: peristome broad, body broad and large.
4. Genus *Balantidium*: parasitic, inhabiting the alimentary tract of the host, body ovoidal or ellipsoidal, blunt at ends in vegetative stage, macronucleus ellipsoidal.
5. The parasite is closely allied to *B. coli* Stein and *B. elongatum* Stein; it differs from them in size of the body and in relative size of the nucleus. No previous record of this form has been found.

I wish therefore to designate this species *Balantidium orchestium*.

Urbana, Ill., June, 1915.

REFERENCES CITED

- Doflein, F. 1911. Lehrbuch der Protozoenkunde. Jena, 1043 pp.
Minchin, E. A. 1912. An Introduction to the Study of the Protozoa. London, 520 pp.

BOOK REVIEWS

REPORT OF FIRST EXPEDITION TO SOUTH AMERICA. Harvard School of Tropical Medicine. Richard P. Strong, Ernest E. Tyzzer, Charles T. Brues, A. W. Sellards, J. C. Gastiaburu. Cambridge. Harvard University Press, 1915. 220 pages. 48 plates.

This very excellent report deals with the investigations of the first expedition sent out by the Harvard School of Tropical Medicine, to study certain forms of tropical disease occurring in South America. The report treats of the sanitary conditions of the places visited, namely, Kingston, Jamaica, Colon, Panama, Buenaventura, Guayaquil, Callao, Lima, and certain mountain towns in the interior of Peru.

While at Guayaquil yellow fever was very prevalent and many cases of this disease were studied. Especial attention was paid to the study of the blood, owing to the reports of Seidlin regarding the presence of a protozoon which he has named *Paraplasma flavigenum* and which he regards as the cause of the disease. As a result of their investigation of the blood in yellow fever, these investigators say: "We were unable, however, to detect any bodies which suggested a parasitic nature in the blood in this disease."

The larger portion of the report deals with the investigations of oroya fever and verruga peruviana and as the results of their investigations the authors have determined, apparently without question, that these conditions are distinct diseases. Verruga peruviana is due to a virus which may be transmitted to animals by direct inoculation, the nature of which is still unknown, while oroya fever is due to a parasite of the red blood corpuscle, which cannot be transmitted to animals. The parasite concerned in the etiology of this disease is of interest to protozoologists because it apparently belongs to a new genus which Strong has called *Bartonella*, naming the genus after Barton, who in 1905 first described the bodies within the red corpuscles. The specific organism causing oroya fever has been named *Bartonella bacilliformis*. Multiple infection of the red cells is common in severe infections, as many as ten of the parasites being present in one cell. Morphologically the parasites occur in the form of minute rods resembling a bacillus, measuring from 1 to 2 microns in length and rounded or oval forms, measuring from 0.3 to 1 micron in diameter. Both rods and rounded forms contain, in stained preparations, granules which may be interpreted as chromatin. The investigators were unable to infect either monkeys or rabbits with this parasite.

While unable to find any parasite in the cases of verruga peruviana studied, the authors were able to produce very typical lesions in both monkeys and rabbits, thus demonstrating the distinct nature of the two diseases. They were unable to definitely decide whether the virus of verruga is filterable, but believe that ultimately it will be shown to be so. As regards the transmission of the two diseases the authors were unable to determine in what manner this occurred but lean to the opinion that some arthropod is concerned.

Uta, a disease of the skin, occurring in Peru, was also studied and the authors were able to demonstrate that this disease is due to a species of *Leishmania*. As regards the species they say: "For the present at least, from the evidence available we do not feel justified in creating a new species for the parasite discovered by us as the etiological factor of uta." A wise decision in view of the present unsatisfactory classification of the Leishmanias.

The report also contains a description of a new Linguatulid parasitic in the crocodile, in Ecuador, which Wheeler has named *Poracephalus crocodili*; a discussion by Brues of the flies of the family Phoridae obtained by the expedition, and some notes on Peruvian mosquitoes and mosquito literature, by Knab.

The book is beautifully printed and illustrated and forms an excellent example of the good work that may be accomplished by medical expeditions. The authors are to be congratulated on the success of their labors for they have contributed a most interesting and valuable addition to the literature and knowledge of tropical medicine.

MEDICAL ZOOLOGY IN BRAZIL.—At present marked attention is being paid to parasitology as is evidenced by recent publications noteworthy in form and character. This movement has found its expression both in monographic articles accorded a place in general medical literature and in a special periodical devoted to the subject. Brief comments on these items will be of service to North American parasitologists who may not have seen the original publications as yet.

Archivos Brasileiros de Medecina (Anno iv, nos. 1, 2, 3) contains an extended discussion of hookworm and hookworm disease covering seventeen separate contributions on all phases of the subject, illustrated by nine full-page plates. The work is complete and scholarly in treatment, giving much that is not available elsewhere. Of especial value is the critical bibliography on ancylostomiasis in Brazil.

Memorias do Instituto Oswaldo Cruz, published at Rio de Janeiro, which reached its seventh volume in 1915, includes original articles on protozoology, helminthology, and medical zoology generally. The text is usually printed in Portuguese and French or German in parallel columns. The journal is illustrated by full page plates in heliotype and lithography in colors, which are very successful and deserve high praise. Most articles concern those elements of the fauna of Brazil which are related to the cause and dissemination of disease. A recent number contains also a sketch of Professor von Prowazek accompanied by a fine portrait.

ARCHIVES DE PARASITOLOGY.—Publiées par Raphael Blanchard, Professeur à la Faculté de Médecine de Paris, Member de l'Académie de Médecine.

Les Archives de Parasitologie, imprimées à Lille (Nord), ont dû suspendre leur publication, par suite de l'occupation de la ville de Lille par les armées allemandes.

Le fascicule du tome XVI porte la date du 1^{er} août 1914. Il était remis au chemin de fer, quand la guerre a éclaté. Il a dû être retiré et, depuis lors, n'a pu être distribué.

Voici son sommaire :

P. S. de MAGALHAES, Notes d'helminthologie brésilienne, dixième série (avec 7 figures dans le texte), page 481.

R. BLANCHARD, Notices biographiques, XXIV. Louis-Daniel BEAUPERTHUY, 1807-1871 (avec 2 figures et 7 fac-simile dans le texte), 503.

G. R. BLANC, Sur quelques espèces du genre *Diplotriana* Railliet et Henry (avec 10 fig. dans le texte), 546.

P. MOLA, Cestodes Avium. Contributo alla fauna elmintologica sarda (pl IX), 557.

Les Parasites et les maladies parasitaires dans l'histoire, la poésie et l'art, 579-637.

Notes et Informations, 638.

Table des matières, 639-640.

Le premier fascicule du tome XVII était en bonne voie d'impression; les cinq premières feuilles étaient déjà tirées, au moment de la déclaration de guerre.

Les *Archives* ne pourront reprendre leur publication régulière qu'après la cessation des hostilités. Elles paraîtront alors par fascicules moins gros et plus rapprochés.

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THE SIGNIFICANCE OF CERTAIN NATURAL FLAGELLATES OF INSECTS IN THE EVOLUTION OF DISEASE IN VERTEBRATES

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INTRODUCTION

During the last few years, considerable attention has been given to the rôle of insects in the spread of disease. Much work has been done in elucidating life-histories, more particularly of the parasitic flagellates peculiar to insects and having no apparent connection with vertebrate maladies. Many flagellates that are seemingly limited to insects, however, are not so innocuous to vertebrates as they appear at first sight. The introduction of certain of the parasitic Mastigophora, notably members of the genera *Herpetomonas* and *Crithidia*, into vertebrates by the latter swallowing the infected insects or by forms of the parasite entering the vertebrate host by way of wounds or abrasions of the skin, has been shown to result in pathogenic effects to the said hosts. Laveran and Franchini have demonstrated the existence of this capacity for exercising latent pathogenicity by infecting dogs with the flagellates of dog fleas, and rats and mice with the flagellates of the fleas infesting these rodents. The present authors, working quite independently of Laveran and Franchini, have conducted a series of experiments extending over some six years on the possible pathogenic effects that accrue when certain flagellates of insects reach either associated or unassociated hosts. We have considered more especially the evolution of disease as exemplified by flagellates that have induced a flagellosis in vertebrates, remembering that, at any rate in some cases, parallel conditions prevail in Nature and in our experiments. By the introduction of certain herpetomonads normally parasitic in insects into vertebrates, a condition resembling leishmaniasis or kala-azar in man

has been produced, the symptoms of the disease and the morphology of the parasites found therein showing that here are, at least, examples of "leishmaniasis in the making."

The evolution of the parasitic habit with the development of pathogenicity as the result of change of habitat is no new phenomenon to those who have carefully studied the comparative morphology and life histories of various pathogenic Protozoa. Change of habitat has frequently led to great alteration in the mode of life of an organism. Thus, when a herpetomonad has so adapted itself that it is capable of living and propagating in a vertebrate, as has probably happened in the case of *Leishmania*, an originally monogenetic parasite by the exertion of its capacity for plasticity becomes digenetic. The various herpetomoniasis, of which the leishmaniasis are a special section, are probably the result of the introduction of insect or other invertebrate flagellates into the vertebrate hosts. In the case of acute forms of disease, the excitants show less perfect power of adaptation to the new environment than do the parasites that induce the chronic type of malady. From the point of view of the parasite, the maintenance of the life of the host is an economic desideratum, the prolongation of the active life of the invader depending in part on the longevity of the host. The newer a parasite is to the animal harboring it, the less it is in harmony with its environment. The consequence is that its discord with the host is manifested by pathogenic effects and the latter animal succumbs. Chronic maladies are usually correlated with greater powers of adaptation of the parasite to its host, with the period that has elapsed since the original introduction of the parasite to the host, and with the relative resisting powers of the host to the specific action of the parasite.

Certain trypanosomes appear to have developed from the flagellates of certain insects (for example *Drosophila*), which parasites in turn seem to have been derived from free-living forms. As free-living organisms, their power for harm appears to be negative. If they become saprophytic, their capacity for developing noxious qualities is increased. When the parasitic habit of life, such as bloodsucking, is established in an insect, the power for injury possessed by its contained flagellates is greatly extended. Finally when the insect flagellate reaches the higher vertebrate the recapitulative effects of its evolution manifest themselves with cumulative results and the parasite is definitely pathogenic. The scale of evolution thus outlined is exhibited by the Trypanosomidae, some of whose members, the trypanosomes and herpetomonads (*Leishmania*) of vertebrates are notably pathogenic. While they are in the insect host, to which they have adapted themselves, they are relatively innocuous.

Yet other Protozoa afford studies in the evolution of pathogenicity. It must suffice to mention the case of the malarial parasites. The

morphology of these organisms shows that they are closely allied to the Coccidia, and there is little doubt that the malaria excitants were originally Coccidia of insects, that with change of habitat developed increased powers of adaptation to life in vertebrates, and, at the same time, increased in pathogenicity towards the new host.

The evolution of disease in the past is presented to us by the example of the malarial parasites. Disease in the making is manifested to mankind today in the case of the herpetomonads more especially. Preventive measures gain in efficacy as the natural modes of infection and possible sources of disease excitants are considered. In this connection, the experiments that we have undertaken may be of service as indicating possible sources of disease hitherto unsuspected.

MATERIAL AND METHODS

The materials used in this research consisted of various flagellates found in insects on the one hand, and of representatives of each of the great phyla of European vertebrates on the other.

The flagellates used in our experiments included members of the genera *Herpetomonas* and *Crithidia*. They comprised *Herpetomonas jaculum* Léger, parasitic in the gut of the Hemipteran, *Nepa cinerea*; *H. stratiomyiae* Fanham and Porter, from the intestine of the Dipteran, *Stratiomyia chameleon*; *H. pediculi* Fanham, from the alimentary tract of *Pediculus vestimenti*; *H. culicis* Novy and MacNeal, from the larvae and adults of the gnat, *Culex pipiens*; and *Crithidia gerridis* Patton, parasitic normally in the alimentary tract of the Hemipteran, *Gerris paludum*.

The vertebrate hosts included representatives of the Pisces (*Gasterosteus aculeatus*), Amphibia (*Rana temporaria*, *Bufo vulgaris* and *Molge vulgaris*), Reptilia (*Lacerta vivipara* and *Tropidonotus natrix*), Aves (*Serinus canarius*, *Passer domesticus* and *Chelidon urbica*) and Mammalia (*Canis familiaris* and *Mus musculus*).

The insect flagellates were introduced into their respective vertebrate hosts either by inoculation or by feeding. In the latter case, the host was fed with the infected insects, or with the intestines of the insects, or with food contaminated with the feces of the insects containing the resistant (nonflagellate or postflagellate) stages of the Flagellata concerned. After the infective feed or feeds, ordinary food was given. The hosts were examined for blood parasites and for ectoparasites prior to use, and were found free from both classes of infestation.

Blood films were made periodically during the life of the infected animals, and smears of the internal organs were prepared at autopsy. Some preparations were fixed while moist with osmic vapor followed by absolute alcohol, while others were fixed wet with Bouin's fluid.

Intravital staining was often employed. For permanent preparations Giemsa's stain, hematoxylin and eosin, iron hematoxylin and occasionally hematein were employed.

Control vertebrates were kept in each case. They remained healthy, lived longer than the experimental animals and were found to be unparasitized when killed.

EXPERIMENTAL WORK

The course of our experiments, extending over some years, on the introduction of insect flagellates into vertebrates may be gathered from the subjoined table. It has not been possible to manage a large number of animals satisfactorily at one time, as some have lived for relatively long periods. Sometimes it was possible to introduce the more resistant, encysted, leishmaniform, postflagellate forms of the *Herpetomonas* or *Crithidia* into the vertebrate. In such cases, it was found that a larger proportion of infections ensued than when the preflagellate or flagellate forms alone were introduced.

It is of some interest to note that as a rule the *Herpetomonas* or *Crithidia* introduced were few in number. The parasites, however, adapted themselves to their new surroundings, and both nonflagellate and flagellate organisms in all stages of active division have been recovered from the infected hosts. In some cases it has been possible to observe the completion of the multiplication in fresh preparations of the organs of the host. The parasites in the vertebrates are not merely conserved. The number of parasites obtained from the vertebrate host is superior to that introduced, multiplication of the organisms occurs and the host often undergoes pathogenic changes resembling those of leishmaniasis, that have frequently resulted in death. The herpetomoniasis induced is therefore regarded as a true parasitic infection, both the causal parasites and the maladies having affinities with kala-azar.

The possibility of bacterial contamination of the material used for feeding or inoculation has not been overlooked. Heavy bacterial contamination was exceptional in the insects used for experiment, and here, as elsewhere, the flagellates often die out in the presence of many bacteria. Further, in Nature pure cultures of flagellates or other protozoa rarely occur, as, for example, is evidenced by the diverse organisms found in sores of man in the East and in the mixed flora and fauna of the alimentary tract of vertebrates. Also, the insects swallowed by such vertebrates as lizards and snakes cannot, of necessity, contain pure cultures of flagellates or bacteria—in fact, mixed infections are frequent in Nature.

The tabular summary of our experiments is given on page 154.

From the following table, it will be seen that while a number of the infections are of the acute type, yet there are others in which the herpetomoniasis induced was of relatively long duration. We may mention that when the infection was of the chronic type, the leishmaniform, nonflagellate bodies preponderated in the smears of the organs of the vertebrate host, while flagellate herpetomonads were more numerous in the cases of acute herpetomoniasis. In practically all the animals infected experimentally, both nonflagellate and flagellate forms of the organism introduced were present, the proportion of each form showing variation. While these conditions prevailed in our experiments, we do not consider that any generalization can yet be made therefrom.

We would also point out that our experiments show the potential danger of many flagellates of insects that may at first sight seem unconnected with the vertebrates into which they have been introduced. Natural modes of infection, however, occur with a number of examples; thus, the dog may contract infection with herpetomonads by eating dog fleas and by ingesting infected flea feces when licking its coat, *H. jaculum* from *Nepa cinerea* can easily reach the fish and amphibia which it attacks and may even reach man by way of the wounds inflicted by the raptorial cutting forelimbs used when the insect sucks blood. The case of insectivorous birds whose normal food is insects is obvious. The experiments, whether between associated or unassociated insect flagellates and vertebrates, show "leishmaniasis in the making."

The chronic infections afford examples of good powers of adaptation to environment on the part of the parasites. As noted in our introduction, it is to the advantage of the newly established organism that the life of the host should be prolonged, and thus the continued existence of the parasite ensured. The acute cases are marked by the rapid development of the flagellate forms of the organisms, and by their less perfect adaptation to new surroundings as manifested in their pathogenic effects to their new hosts.

A further point of interest is that when young hosts were used, the parasites were more virulent. This is also the case with the parasites causing the human disease, Mediterranean kala-azar, which is prevalent more especially in children.

MORPHOLOGY OF THE PARASITES IN THE VERTEBRATE AND INVERTEBRATE HOSTS

The life-history of a herpetomonad in its insect host may be briefly outlined as follows: A *Herpetomonas* is a flagellate possessing also a nonflagellate stage in its life-cycle. This nonflagellate form is an ovoid or rounded, leishmania-like body containing a nucleus and a blepharoplast. It (Fig. 1a) may be passed from the host with the feces of the

TABLE—RESULTS OF THE EXPERIMENTAL INFECTIONS OF DIFFERENT VERTEBRATES WITH VARIOUS HERPETOMONAS AND CRITHIDIA FROM INSECTS

No. of Experiment	Vertebrate Host	Flagellate Introduced	Mode of Introduction	Duration of Life of Host	Effect on Host	Forms of Parasites Observed in the Vertebrates	Remarks
1	Wild mouse, <i>Mus musculus</i> , ♀	<i>Herpetomonas jaculium</i>	Feeding.....	50 hours	Acute herpetomoniasis	Flagellate and non-flagellate	Young host
2	Wild mouse, ♂	<i>Herpetomonas jaculium</i>	Feeding.....	70 hours	Acute herpetomoniasis	Flagellate and non-flagellate	Young host
3	Wild mouse, ♀	<i>Herpetomonas jaculium</i>	Intraperitoneal inoculation	60 hours	Acute herpetomoniasis	Flagellate and non-flagellate	Young host
4	Wild mouse, ♂	<i>Herpetomonas jaculium</i>	Feeding.....	60 hours	Acute herpetomoniasis	Flagellate and non-flagellate	Host killed in extremis
5	Wild mouse, ♀	<i>Herpetomonas jaculium</i>	Feeding.....	84 hours	Acute herpetomoniasis	Flagellate and non-flagellate	Host killed when very ill
6	Wild mouse, ♂	<i>Herpetomonas jaculium</i>	Intraperitoneal inoculation	72 hours	Acute herpetomoniasis	Flagellate and non-flagellate	Host killed in extremis
7	Adult mouse, ♂	<i>Herpetomonas jaculium</i>	Intraperitoneal inoculation	Killed after 8 months	No symptoms of disease	Few non-flagellate..	Spontaneous cure, no parasites found at autopsy
8	Mouse, ♂	<i>Herpetomonas stratiomyiae</i> ..	Feeding.....	5 days.....	Herpetomoniasis induced	Nonflagellate and some immature flagellate	Young host
9	Mouse, ♀, adult	<i>Herpetomonas pediculi</i>	Feeding.....	72 days.....	Herpetomoniasis induced	Mostly nonflagellate, very few flagellate forms	Chronic infection
10	Mouse, ♀, adult	<i>Herpetomonas pediculi</i>	Fed on liver of No. 9	15 days.....	Herpetomoniasis induced	Non flagellate and flagellate	
11	Mouse, ♀, adult	<i>Crithidia gerriidis</i>	Intraperitoneal inoculation	40 days.....	Infection with <i>C. gerriidis</i> induced	Flagellate and non-flagellate forms, the latter more numerous	Killed in extremis. Skin sore and abscess at the site of inoculation
12	Mouse, ♀, adult	<i>Crithidia gerriidis</i>	Subcutaneous inoculation	2 months, then killed	Negative		
13	Mouse, ♂, adult	<i>Crithidia gerriidis</i>	Feeding.....	38 days.....	Infection with <i>C. gerriidis</i> induced	Flagellate and non-flagellate, the latter predominating	Young host. Spontaneous cure. No parasites found when killed
14	Dog, <i>Canis familiaris</i> , ♂	<i>Herpetomonas etenocephali</i> ..	Feeding.....	Killed after 15 months	No marked permanent ill-effects	Nonflagellate	

15	Canary, <i>Serinus canarius</i> , ♀, adult	<i>Herpetomonas jaculum</i>	Feeding.....	51 days.....	Chronic herpetomoniasis induced	Many nonflagellate, a few flagellate	Note chronic infection probably to be correlated with the presence of nonflagellate forms
16	Sparrow, <i>Passor domesticus</i> , ♀, adult	<i>Herpetomonas culicis</i>	Feeding.....	9 days.....	Acute herpetomoniasis induced	Flagellate and nonflagellate, the former predominating	Note acute infection, probably to be correlated with the presence of many flagellate forms
17	Martin, <i>Chelidon urbica</i> , ♀, young adult	<i>Herpetomonas culicis</i>	Feeding.....	12 days.....	Acute herpetomoniasis induced	Flagellate and nonflagellate, the former dominant	Note acute infection and many flagellate forms present
18	Martin, ♀, young adult	<i>Herpetomonas culicis</i>	Subcutaneous inoculation	2 days.....			Probably died of fright
19	Canary, ♂, young...	<i>Herpetomonas jaculum</i>	Feeding with infected insect excrement	17 days.....	Herpetomoniasis induced	Flagellate and nonflagellate	
20	Martin, ♂, mature young	<i>Herpetomonas culicis</i>	Feeding with infected insect excrement	32 days.....	Herpetomoniasis induced	Nonflagellate and a few flagellate	
21	Sparrow, ♀	<i>Herpetomonas jaculum</i>	Feeding with infected insect excrement	Killed after 3 months		One parasite only seen during life	Spontaneous cure. No parasites found at autopsy
22	Canary, ♀, adult...	<i>Herpetomonas culicis</i>	Fed on food contaminated with <i>H. culicis</i>	Killed after 80 days	Negative		
23	Grass snake, <i>Tropidonotus natrix</i> , ♂	<i>Herpetomonas jaculum</i>	Feeding.....	20 days.....	Herpetomoniasis induced	Flagellate and nonflagellate	
24	Lizard, <i>Lacerta vivipara</i> , ♂	<i>Critidia gerriidis</i>	Feeding.....	19 days.....	Infection with <i>C. gerriidis</i> induced	Flagellate and nonflagellate	
25	Lizard, ♂	<i>Critidia gerriidis</i>	Fed on infected liver of No. 24	6 days.....	Acute disease (critidiasis)	Flagellate and nonflagellate	Second passage
26	Lizard, ♀	<i>Critidia gerriidis</i>	Intraperitoneal inoculation with infected heart blood of No. 25	Killed after 20 days	Slight infection	Nonflagellate	Killed for examination after 20 days. Third passage
27	Frog, <i>Rana temporaria</i> , ♂, adult	<i>Critidia gerriidis</i>	Intraperitoneal inoculation	29 days.....	Infection with <i>C. gerriidis</i> induced	Flagellate and nonflagellate	
28	Frog, ♂, adult.....	<i>Herpetomonas jaculum</i>	Intraperitoneal inoculation	54 days.....	Herpetomoniasis induced	Flagellate and nonflagellate	
29	Toad, <i>Bufo vulgaris</i> , ♂, adult	<i>Herpetomonas jaculum</i>	Subcutaneous inoculation	40 days.....	Chronic infection	Nonflagellate and young flagellate	
30	Toad, ♀, adult.....	<i>Herpetomonas jaculum</i>	Intraperitoneal inoculation	80 days.....	Negative	None	
31	Newt, <i>Molge vulgaris</i> , ♂, young adult	<i>Herpetomonas jaculum</i>	Feeding.....	9 days.....	Apparently negative	None	Death by misadventure
32	Stickleback, <i>Gasterosteus aculeatus</i> , ♂	<i>Herpetomonas jaculum</i>	Feeding.....	2 days.....		None	
33	Stickleback, ♀	<i>Herpetomonas jaculum</i>	Subcutaneous inoculation	6 days.....	Herpetomoniasis induced	Flagellate and nonflagellate	

latter, and is then surrounded with an outer coat. If the excrement containing the nonflagellate—sometimes termed encysted or postflagellate—forms of the herpetomonad is ingested by another insect host, these ovoid forms of the parasite have their firm, varnish-like outer coat (Fig. 1f) dissolved by the digestive juices of the host and are then capable of further development. In this condition, they are often termed preflagellate forms (Fig. 1a). The preflagellate form gradually elongates. A flagellum arises near the blepharoplast (Fig. 1c), reaches the surface of the body at the anterior end and finally projects as a free flagellum. The posterior end also elongates and thus the typical flagellate is produced (Fig. 1d).

Multiplication of the flagellate by longitudinal division can occur in either the nonflagellate (Fig. 1b) or the flagellate stage (Fig. 1e). As the organisms pass onward into the less favorable environment of

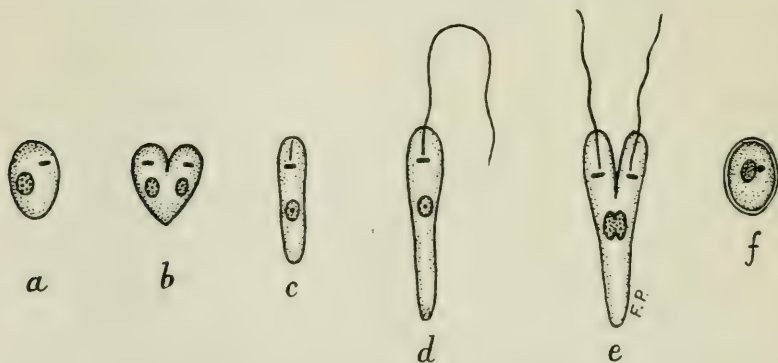


Fig. 1.—*Herpetomonas*: (a) non-flagellate or leishmaniform stage; (b) dividing non-flagellate; (c) elongating parasite; (d) flagellate stage; (e) dividing flagellate; (f) post-flagellate or encysted stage. $\times 1500$.

the posterior end of the intestine of their host, their body cytoplasm concentrates, and the flagellum is withdrawn and largely dissolved. The now ovoid parasite secretes a coat which may be at first gelatinous but ultimately becomes varnish-like or "skin tight" and the postflagellate form is again produced. This resistant nonflagellate form (Fig. 1f) is particularly adapted for extracorporeal life and serves for the safe transference of the parasite from host to host.

The above outline of the life history of a herpetomonad is valid for *Herpetomonas jaculum*, *H. stratiomyiae*, *H. pediculi*, *H. culicis* and *H. ctenocephali* with which we experimented.

The life-history of a true *Crithidia*, such as *C. gerridis*, in its insect host has the same general outline as that of a *Herpetomonas*. But the flagellate stage differs from that of a *Herpetomonas* in that at the differentiation of a flagellum, this structure not only reaches the sur-

face, but forces the ectoplasm before it, thus producing a small wavy undulating membrane that gradually fades into the free flagellum at the tapering anterior, flagellar end of the body of the organism (Fig. 2b).

The morphology of *Herpetomonas jaculum*, *H. stratiomyiae*, *H. pediculi*, *H. culicis* and *H. ctenocephali* in the vertebrate hosts into which they were introduced resembled that in the invertebrate hosts. The parasites have been introduced both as flagellates and as nonflagellates. In blood smears taken during the life of the host and in organ smears made at autopsy, usually both flagellate and nonflagellate forms were found. Parasites in various stages of multiplication were

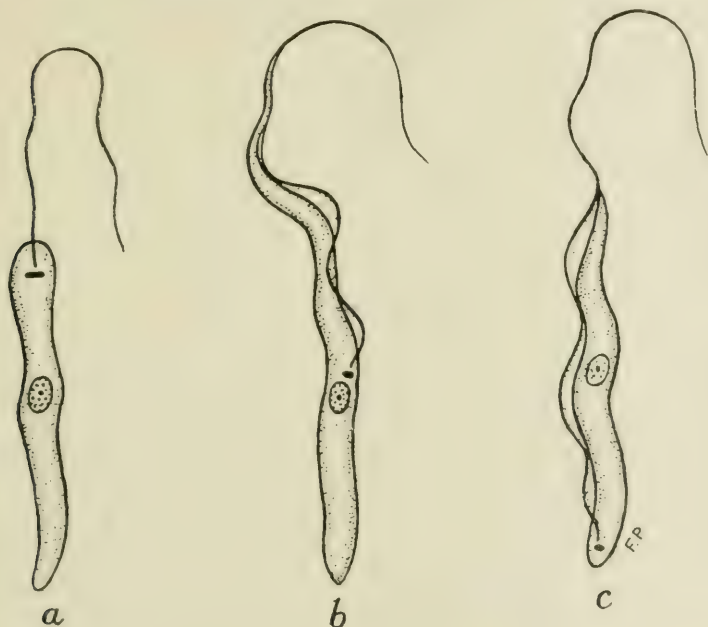


Fig. 2.—Flagellate forms of (a) *Herpetomonas* (sometimes called *Leptomonas*), (b) *Crithidia* and (c) *Trypanosoma*. $\times 2000$.

observed in the fresh condition and in stained preparations. Hence there is definite evidence that they had become true parasites of the vertebrates, had established themselves and had increased in numbers in them, and were not mere conservations of the forms introduced.

The various *Herpetomonas* (Fig. 2a) and *Crithidia* (Fig. 2b) that we have used have retained the facies that they presented in the insect hosts. No transition to a trypanosome (Fig. 2c) was ever seen by us during the course of these experiments. The only variation presented by the parasites in the vertebrates from that in the invertebrates was that the maximum length of the insectan flagellate stage was not usually quite attained. The sizes of the parasites, however, were

always well within the range of the limits of variation given for the insect parasites and were of good average size. Morphologically, they were replicas of the insect forms and could be unmistakably identified with them. The nonflagellate forms were about the size of or slightly greater than the Leishman-Donovan body in man, while the dimensions of the flagellate forms were much the same as those of *Leishmania* in cultures on the Novy-MacNeal-Nicolle medium, that is, about 15μ to 20μ in the long diameter of body. The slightly lesser dimensions of the parasites may be the results of transference and implanting of the organisms in new hosts, or perhaps the age of the host may influence the size of the parasite. It has been noticed that other parasites, for instance, certain Haemosporidia, introduced into unfamiliar vertebrates or into young hosts tend to produce new generations whose maximum dimensions are somewhat less than those of their progenitors. The same factors may apply in this induced herpetomoniasis. On the other hand, it is known that the nonflagellate parasite of Indian kala-azar maintained in dogs may increase in longest diameter from about 2.5μ or 3.5μ to 8μ or 9μ . Similar variations in size occur in the non-flagellate stages of closely allied herpetomonads in insects.

THE SIGNIFICANCE OF CERTAIN NATURAL FLAGELLATES OF INSECTS IN
THE EVOLUTION OF DISEASE

The rôle of insects in the spread of disease among men and other animals has furnished some of the most important advances in knowledge made in recent times. Many parasitic protozoa are the descendants of free-living ancestors. The degrees of degradation from independent life to saprophytism and thence to parasitism are almost imperceptible but nevertheless exist. Neither are the grades of parasitism more well defined, and consequently a free-living organism that by accident or chance has reached the alimentary tract of an insect may live there first as a saprophyte, feeding on the waste materials or the newly ingested food of the host. The minute quantities of nourishment lost by the host in this way become serious when cumulative, and the saprophytism then leads to parasitism of a somewhat low degree. When the living protoplasm of the host furnishes the nutriment required, the parasitism becomes obvious and the effects on the host are more or less marked.

In the case of many intestinal flagellates of insects, the host has responded to the attacks of the parasites in such a way that a mutual toleration has become established between them. Under these circumstances but little injury ensues to the host, and the flagellates concerned are considered as "natural" and practically harmless to the host. Further, they have often been considered as specific to the said hosts to which they are practically harmless.

Should such flagellates reach a vertebrate host, two courses may result. In the first instance, the flagellates may merely perish. In the second case, should the introduced organism be sufficiently plastic, it may adapt itself to its new environment and be able to persist for a time. Should its powers of adaptation be marked, it will multiply, and the greater the rapidity of increase, the greater the danger to the host. In other words, environment and plasticity determine pathogenicity.

Certain of the flagellates show the transformation from almost harmlessness in the insect to pathogenicity in the vertebrate or newer host. The genus *Herpetomonas* affords a good example of the capacity for pathogenicity that may be latent in many organisms hitherto considered harmless.

Kala-azar, oriental sore and dermomucosal leishmaniasis are well-known tropical diseases due to members of the *Herpetomonadidae* that are known as *Leishmania donovani*, and *L. infantum* in the cases of kala-azar, and as *L. tropica* in the more local maladies of the skin. These organisms are, in all probability, herpetomonads of insects that have reached vertebrate hosts. It is known that the various species of *Leishmania* develop into typical herpetomonad flagellates in cultures, and for some time now these flagellate stages have been known in man. Thus, in 1911, Escomel saw flagellate forms of *Leishmania tropica* in man and published about them later. La Cava in 1912 described similar forms of the same parasite in Italy. Also in 1912, Splendore found elongating forms and a few flagellate parasites in dermomucosal leishmaniasis in Brazil, while Monge in 1914, when working on the same malady in Peru, found the herpetomonad stage of the parasite. Lately (September, 1915) Wenyon has found the flagellate stage of *Leishmania donovani* in a dog'subinoculated from other dogs, the strain being originally derived from a man who died of kala-azar contracted in Calcutta. Further, a new herpetomonad, *Haemocystozoon brasiliense*, was found by Franchini in 1913 in a human subject.

As a result of experimental work, such as that of Patton and of Wenyon, it has been shown that species of *Leishmania* can develop into herpetomonad flagellate stages within the intestines of certain insects, such as bedbugs and mosquitos (*Stegomyia*). The evidence that an ovoid *Leishmania* is the non-flagellate stage of a herpetomonas is proved, and the flagellate stage of *Leishmania* can exist in cultures, in insects and in man. *Leishmania* morphologically is a herpetomonad.

Herpetomonads experimentally introduced into vertebrates by us have produced pathogenic effects recalling those of kala-azar. Both maladies present the same features—the insidious onset, the subsequent relatively rapid illness, the splenic and often hepatic enlargement, feverish attacks and emaciation. In the cases where chronic infections

were produced in our animals, the nonflagellate, leishmaniform stages of the parasites were more numerous, while in acute cases the flagellate forms were more obvious (see table). In the diseases due to *Leishmania* spp. the flagellate forms in the vertebrate host are far less common than the nonflagellate ones, but it is of distinct interest to note that Monge (1914) suggested that the presence of flagellate forms of *L. tropica* in man was an indication of increased virulence on the part of the parasite. Such an increased virulence certainly coincided with more marked development of flagellates in our animals. Though no general conclusion on the subject can yet be given, the hypothesis that the presence of flagellate *Herpetomonas* or *Leishmania* in the vertebrate host affords an index of virulence is supported by the experimental results that we have obtained.

The part played by vertebrates proved capable of harboring herpetomonads is one that demands the attention of all students of preventive medicine and of sanitary reform. By experiment we have proved that flagellates belonging to the genera *Herpetomonas* and *Criethidia* have produced infections not only in mammalia like mice and dogs, but also in birds and in cold-blooded vertebrates such as members of the pisces, amphibia and reptilia. Further, these flagellates are capable of assuming resting, nonflagellate stages in these hosts.

There is thus the possibility that various vertebrates—fish, amphibia, birds, reptilia, and mammals—may serve as reservoirs of the herpetomoniasis, including leishmaniasis. The virus may be very attenuated and so escape detection, or only be revealed by the presence of flagellate forms in cultures. Recently (1914) Sergeant, Lemaire and Senevet in Algeria have demonstrated the presence of a herpetomonad flagellate in the blood and organs of geckos obtained from areas in Algeria in which oriental sore due to *L. tropica* is present. *Phlebotomus* flies, which may harbor a natural herpetomonad, feed on the geckos and on man. Hence animals like geckos may possibly act as reservoirs of leishmaniasis. Chatton and Blanc (1914) have found possible leishmaniform bodies in the young red blood cells of geckos in Tunis. Bayon (1915) has found herpetomonad parasites in the cloaca of *Chameleoni pumilus* at Robben Island, South Africa, and says that "it does not seem excluded that a chameleon can get infected through swallowing a fly containing *Herpetomonidae* in its gut." He also found a herpetomonad in the gut of the fly, *Scatophaga hottentota*, in the same place. Lindsay (1914) stated that the parasite of dermomucosal leishmaniasis in Paraguay is believed by native sufferers to be conserved in rattlesnakes and to be spread by ticks or flies (*Simulium*) feeding on the reptiles and transferring the parasite to man. We have shown the possibility of such infection occurring by causing insectivorous vertebrates, such as viviparous lizards and grass snakes, to

ingest insects infected with herpetomonads, wherewith the vertebrates became parasitised. Similarly, insectivorous birds have become parasitised by ingesting insects containing herpetomonads. These infections could be accomplished in Nature and, in fact, such parasitism of a bird by herpetomonads and of mice by the same flagellates has been found (see below). Natural reservoirs of herpetomoniasis, consisting of vertebrates on which sanguivorous insects feed, should be sought for in areas where diseases such as kala-azar are present.

Natural reservoirs of herpetomoniasis are already known. Man and his intimate domestic associate the dog, both may function as reservoirs of what has been termed Mediterranean or infantile kala-azar. The parasite, *Leishmania infantum*, which is often considered to be a form of *L. donovani*, is thought to be transmitted from dog to dog by the dog flea and possibly also from dog to man. An infected child or an infected dog may, perhaps, serve as the reservoir of the virus. In this connection it is of some interest to recall that cattle which have become immune to piroplasmiasis may yet harbor sufficient sparse piroplasms in their blood to infect many ticks and so spread the malady. Analogy is somewhat dangerous, but in this case, it may be of service, since rare cases of "spontaneous cure" of infantile leishmaniasis are known and it is just possible that such may act as unsuspected reservoirs of leishmaniasis.

Vertebrates other than man can be infected naturally with herpetomonads. In 1903, Dutton and Todd described herpetomonads from the blood of house mice in Senegambia. The original description was very definitely that of a *Herpetomonas*, though Todd has recently stated that he thinks the organism may have been a trypanosome. However, we have also found herpetomonads closely resembling those described by Dutton and Todd in mice in England. It is known that the common rat-fleas contain herpetomonads and it is suggested that these fleas were the probable source of infection. Mice as possible reservoirs of leishmaniasis cannot be disregarded.

Again, a natural infection of birds has been described by Drs. Edmond and Etienne Sergent. In this case a pigeon was found to contain herpetomonads in its blood. The source of the flagellate is not known with certainty, but we advance the hypothesis that it was a latent herpetomoniasis contracted from herpetomonad-infected insects such as species of *Lynchia* that had fed on the bird.

From a careful comparison of natural and induced herpetomoniasis in vertebrates and of leishmaniasis, as well as consideration of the morphology and life phenomena of the excitants in each case, the following general statements can be made. Under suitable conditions, insect flagellates can be introduced into vertebrate hosts and can produce infections therein. In some cases, as in some cold-blooded verte-

brates, little obvious ill effect results; in others, as in mammals and birds, disease is manifested and often ends in death.

The organisms, such as herpetomonads, thus introduced, retain their powers of development on the same lines as when they were present in the insects. The morphological cycle is that of *Herpetomonas*. The various species of *Leishmania* are probably insect flagellates long since introduced into man and usually perpetuating the nonflagellate form, though capable of assuming the flagellate, herpetomonad facies in the internal organs of the vertebrate or in the invertebrate hosts.

No insect flagellate can be considered to be quite innocuous to vertebrates until it has been put to the test.

It must be remembered that leishmaniasis, which is a form of herpetomoniasis, is a flagellosis, as is also trypanosomiasis. The treatment of leishmaniasis by intravenous injection of tartar emetic—as advocated and practiced recently—is sound biologically, for drugs containing arsenic or antimony have proved efficacious in trypanosomiasis.

It is necessary to consider not part, but the whole, of the life history of an organism and also the relationship of the parasite to the group to which it belongs. There is a line of evolution common to each group and in these cases, neither *Herpetomonas* (*Leptomonas*), *Leishmania*, *Crithidia* nor *Trypanosoma* (Fig. 2) should be considered as isolated units but as flagellates belonging to the Trypanosomidae.

MODES OF INFECTION AND PREVENTIVE MEASURES AGAINST ARTHROPOD-BORNE HERPETOMONIASES

The experiments on the introduction of various species of *Herpetomonas* and *Crithidia* parasitic in insects into both warm and cold-blooded susceptible vertebrates has shown that these flagellates can produce an infection in the vertebrates when the latter are fed or inoculated with them. Within the host, the parasite is capable of assuming the leishmaniform or flagellate facies. The mode of infection of the vertebrate in nature seems to be contaminative, either by its food, or through an already existing abrasion or puncture on the surface of its body. The feces of insects, if containing the resistant forms of the flagellate, are capable of producing infection by similar channels. We have also obtained evidence showing that postflagellate forms of the parasite are the best adapted to begin life in a new vertebrate host.

Experiments on ourselves with fleas and lice, and with biting insects on rats, suggest that infection with *Herpetomonas* or *Leishmania* is not by inoculation with the protozoal parasites during the time when the insect is biting man or other vertebrate, but by the vertebrate eating the infected insect, or by infected insect feces passing through an abrasion, puncture or bite on the vertebrate skin. In this connection it is of

interest to note that Laveran has quite recently succeeded in infecting a mouse with a culture of *Leishmania tropica* by way of the mouth.

As we have already stated, in areas where leishmaniasis are endemic, an examination should be made of all insects and other invertebrates likely to come into contact with men or dogs or rats and mice, in order to ascertain if these invertebrates harbor herpetomonads. Preventive measures should be directed against such invertebrates, especially arthropods. Further, it is likely that certain vertebrates, such as reptiles and amphibia (especially such as are insectivorous), may serve as reservoirs for leishmaniasis or, as they should preferably be termed, herpetomoniasis. From such reservoirs the herpetomonads may reach man by the agency of ectoparasites or flies, especially such as are sanguivorous.

That some of these suggestions are of practical application has been proved by the work of Dodds Price in the Assam tea gardens, following on a suggestion from Rogers to the effect that action should be taken against suspected transmitters of kala-azar, even if complete inculcation of them had not been afforded. Dodds Price has reduced the mortality due to kala-azar enormously by segregating the infected, by moving coolie lines about three hundred yards from older, infected ones and by having new coolie lines placed on clean sites. Young (1914) has applied successful segregation measures to an indigenous population in certain villages in Assam. These measures check the prevalence of sanguivorous insects that infest man and his dwellings, and reduce the danger of possible infection by way of contaminated food or drink. It may be expected that the application of similar measures in other areas where kala-azar is endemic may also be equally efficacious.

SUMMARY

1. Herpetomoniasis can be induced in various warm and cold-blooded vertebrates when the latter are inoculated or fed with herpetomonads occurring in the digestive tracts of various insects. The infection produced and the protozoal parasites found in the vertebrates resemble those of human and canine leishmaniasis.

2. An infection can also be induced in certain vertebrates when they are fed or inoculated with *Crithidia gerroidis*, and both flagellate and nonflagellate stages occur therein, but no transition to a trypanosome was found.

3. The following Flagellata have been proved pathogenic to warm-blooded vertebrates when the latter have been fed, or inoculated subcutaneously or intraperitoneally with them—*Herpetomonas jaculum*, *H. stratiomyiae*, *H. pediculi*, *H. ctenocephali*, *H. culicis* and *Crithidia gerroidis*. The hosts used were mice of various ages, dogs, canaries, sparrows and martins.

4. *Herpetomonas jaculum* and *Crithidia gerridis* have also been successfully fed or inoculated into cold-blooded hosts, namely, fishes (*Gasterosteus aculeatus*), frogs, toads, lizards (*Lacerta vivipara*) and grass-snakes (*Tropidonotus natrix*).

5. The disease induced may run an acute or a chronic course. In the acute cases among our vertebrates the flagellate form of the parasite was the more obvious at death. In chronic cases, non-flagellate forms of the parasite were more numerous.

6. Natural herpetomoniasis of a pigeon has been recorded by Drs. Edm. and Et. Sargent in Algeria. This affords a parallel case with the natural and induced herpetomoniasis of mice as recorded by us.

7. The flagellate stage of *Leishmania donovani* in vertebrates is now known, and that of *L. tropica* in man has been known for some time. The links completing the evidence that a *Leishmania* is morphologically a *Herpetomonas* are thus complete. We believe that leishmaniasis are invertebrate-borne herpetomoniasis, and that these maladies have been evolved from flagellates of invertebrates (especially herpetomonads of insects), which have been able to adapt themselves to life in vertebrates.

8. In areas where leishmaniasis are endemic an examination should be made of all insects and other invertebrates likely to come into contact with men or dogs or domestic vermin like rats and mice, in order to ascertain if these invertebrates harbor herpetomonads. Preventive measures should be directed against such invertebrates, especially arthropods. Further, it is likely that members of all classes of vertebrates, and especially those members that are insectivorous, may serve as reservoirs for leishmaniasis, or as they should preferably be termed, herpetomoniasis. The virus may exist in such reservoirs in a very attenuated condition and so be difficult of detection. From these sources the herpetomonads may reach man by the agency of ectoparasites or flies, especially such as are sanguivorous.

ADDENDUM

As this paper—the writing of which has been greatly delayed by war work—was on the point of being despatched, our attention was drawn to an article on The Insect Vector of Uta by C. H. T. Townsend in the December number of the *Journal of Parasitology*, just received in England. The concluding paragraph of the text and more particularly of the summary of Townsend's paper were read by us with very great interest, as they confirm our conclusions regarding leishmaniasis being arthropod-borne herpetomoniasis. This conclusion of ours has met with considerable opposition at the hands of Wenyon, much to our

surprise, and in spite of the fact that the experiments of Laveran and Franchini, as well as the much more extended series of our own, admit of no other conclusion to our mind.

The following conclusions of ours may be compared with those of Townsend (December, 1915). Thus, in November, 1914, we stated that, "It may be expected that the various leishmaniasis, occurring in different parts of the world, will prove to be insect-borne herpetomoniasis." Again, in May, 1915, we wrote that: "As we have previously stated, we believe that leishmaniasis are arthropod-borne herpetomoniasis, and that these maladies have been evolved from flagellates of invertebrates (especially herpetomonads of insects), which have been able to adapt themselves to life in vertebrates." Further, one of us in June, 1915, wrote that: "It is inferred that the various leishmaniasis are due to a herpetomonad of invertebrates which, under different conditions of environment, produces pathogenic effects in very varying degrees in different vertebrates, from zero, as in the mice described by Dutton and Todd in 1903, to high mortality as in Indian kala-azar, and probably zero again in cold-blooded hosts. It is also a flagellate which can probably live in invertebrates not already recorded as being infected. A human reservoir of leishmaniasis may occur in some places, while warm and cold-blooded vertebrates may also function as the same."

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A REVISION OF THE GENUS ARHYTHMORHYNCHUS

WITH DESCRIPTIONS OF TWO NEW SPECIES FROM NORTH
AMERICAN BIRDS *

H. J. VAN CLEAVE

INTRODUCTION

When Lühe created the genus *Arhythmorhynchus* (1911:47) he assigned to it but one species *A. frassoni* (Mol.). The following year in publishing the results obtained from a study of four immature specimens of *Echinorhynchus invaginabilis* von Linstow Lühe (1912:283) ascribed that species to the genus *Arhythmorhynchus* and in the same article accepted two American species, *Echinorhynchus uncinatus* Kaiser and *E. trichocephalus* R. Leuckart, as agreeing with his definition of the genus *Arhythmorhynchus*. Of these four species belonging to this genus but one is well known, namely, *A. frassoni* (Mol.). For the two American species not even the host is known, and while Kaiser (1893) has given minute details regarding the hooks of these two species, data concerning the embryos and many other points which are essential for a complete specific diagnosis are entirely wanting. Consequently it seems that concerning some of the points in the definition of the genus data are available for a single species only. It is not surprising that a generic diagnosis based upon the study of a very small number of species might later require emendation to permit including within the same genus species of obviously close relationship. Especially is this true in groups of parasites, such as the *Acanthocephala*, in which the organization of the body has been reduced to its simplest terms through perfect adaptation to the parasitic existence; for this same reduction eliminates large groups of organs and structures which in nonparasitic forms afford additional characteristics of diagnostic value.

Recently the writer (Van Cleave, 1913) found it advisable to emend the definition of the genus *Neoechinorhynchus* to permit including within it five species which were unknown to the founder of the genus. Similarly now after a study of new materials including two new species closely related to *Arhythmorhynchus frassoni* (Mol.) and *A. invaginabilis* (von Linst.) the writer has found it imperative to modify Lühe's original description of the genus *Arhythmorhynchus* (Lühe, 1911:47) to prevent exclusion from this genus of forms which under a natural

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system of classification could not be granted independent generic rank. The materials upon which the present study has been made were collected by Mr. Albert Hassall and deposited in the Collections of the U. S. Bureau of Animal Industry. Both species are represented in the collection by numerous fully mature individuals so that a complete study of the specific characteristics has been possible in both species. These new forms were found to deviate from Lühe's description of the genus *Arhythmorhynchus* in the following particulars: (1) the shape of the body; (2) the location of the testes with reference to the two regions of the body proper described by Lühe; (3) the shape of the membranes surrounding the hard-shelled embryos in the body cavity of the female. In the estimation of the writer these are placed in the order of their relative significance, the least significant first.

As Lühe has pointed out, the anterior region of the body in members of the genus *Arhythmorhynchus*, which in some species is an inflated oval region standing out in contrast to the smaller cylindrical posterior region (Figs. 1 and 4) contains relatively large numbers of subcuticular nuclei while the posterior region is devoid of subcuticular nuclei. Simple body shape has been so long recognized as a variable quantity by those working with *Acanthocephala* that little emphasis may justly be given it alone. However, when body shape has as an accompanying feature distinctive structural characteristics emphasis may be placed upon the structure as of diagnostic value though broad range of variation may occur in the gross outer form in which the structure finds expression. Therefore, in defining the genus *Arhythmorhynchus*, emphasis should be placed upon the difference in structure between anterior and posterior regions of the body rather than upon the difference in shape of these two regions, for the structure is constant in all species which have been examined though the body form is widely variable. For the males of this genus Lühe has specified that the testes occur in the anterior swollen region of the body (Fig. 1). Suspended as they are in the genital ligament running backward through the body cavity from the base of the proboscis sheath and with no intimate relationship to the body wall little of generic value may be placed upon the exact location of these organs in the body cavity. In one species at least (Fig. 4) the testes do not lie in the swollen region of the body as indicated in Lühe's diagnosis of the genus, but are located distinctly posterior to that region. The third point of difference, the shape of the embryonic membranes, presents the most radical point of divergence in the species under consideration from the original description of the genus. Lühe throughout his classification of the *Acanthocephala* has emphasized the importance of shape of the embryos and structure of their coverings as of marked diagnostic value. For *Arhythmorhynchus* he has specified in his characterization of that genus the presence of

three fully concentric membranes surrounding the embryo within the body cavity of mature females. His observations upon the embryos of *A. frassoni*, the only species of the genus for which sexually mature individuals were at that time known, corroborated the earlier record of de Marval (1904, Fig. 55) for the embryos of the same species. However in *A. brevis* and *A. pumilirostris* the writer has found two species which, though agreeing with Lühe's definition of the genus in all other essential characteristics, present a marked contrast in the structure of the embryos. In each of these species numerous fully mature females have been examined with the unvarying result of disclosing hard-shelled embryos in which the middle membrane has an outpocketing at each pole (Figs. 10 and 12).

In view of the foregoing details, wherein the two newly described species fail to agree with the original definition of the genus, two possibilities present themselves: either (1) a new genus should be established for these two species; or (2) the definition of the genus *Arhythmorhynchus* should be modified so as to include these forms. To the writer it seems unwise to create a new genus for forms which differ from an existing genus by but a single point of essential distinction: namely the shape of the membranes surrounding the embryos. Especially does this seem uncalled for in the case under consideration in which up to the present time, embryos were known for but a single species. Therefore it appears expedient to recast the definition of the genus *Arhythmorhynchus*.

REVISED DIAGNOSIS OF THE GENUS ARHYTHMORHYNCHUS

Acanthocephala with a spindle-shaped proboscis upon which the hooks are arranged not in radial but in bilateral symmetry since those on the dorsal and ventral surfaces of the same individual differ, though in varying degrees in different species. Anterior region of body sharply differentiated from posterior region in structure of body wall, especially in the presence of nuclei in the subcuticula of anterior region only. Portion of the anterior region of body proper spined. Spines entirely wanting on neck and on posterior region of body proper. Proboscis sheath a double-walled muscular sac inserted at the base of proboscis. Central nervous system near center of proboscis sheath. Cement glands very long, slender. Embryos in body cavity of female elongated oval with three membranes, all concentric or the middle one with an outpocketing at each pole. Sexually mature in the intestine of birds.

ARHYTHMORHYNCHUS BREVIS NOV. SPEC.

Body in both sexes with distinct oval enlargement comprising about anterior half. Posterior end distinctly smaller, elongated, cylindrical. Females 6 to 12 mm. long; maximum thickness 3 mm.; diameter of

posterior region about 1 mm. Males, 5 to 6 mm. long; maximum thickness, 1 to 1.5 mm.; diameter of posterior region, 0.5 to 0.75 mm. Neck naked, retractable, tapering toward proboscis, not sharply set off from body, 0.35 to 0.55 mm. long. Body for short distance just back of neck irregularly set with small number of spines 0.012 mm. long. Proboscis elongated with conspicuous expansion near center, 0.665 mm. long, 0.230 mm. in diameter at base, 0.190 mm. at tip, 0.340 mm. at center. Proboscis armed with eighteen longitudinal rows of hooks, usually fifteen in a row. Basal hooks nearly straight, slender, 0.047 mm. long. Heaviest hooks near middle of proboscis 0.041 to 0.047 mm. long, on ventral surface slightly larger than on dorsal. Hooks at tip slender, recurved, 0.047 mm. long. Cement glands long, narrow. Testes oval, slightly overlapping one another, in swollen part of body. Embryos 0.076 to 0.100 mm. by 0.024 to 0.030 mm. Middle of three shells of embryos heavy, with a rounded swelling at each pole. Host *Botaurus lentiginosus* (Montag.), intestine. Type locality Baltimore, Md., U. S. A.; Cotypes in collection Bureau of Animal Industry, Washington, D. C., Catalog No. 6302; and in the Helminthological Collection of the Department of Zoology, University of Illinois, Urbana, Catalog No. 16, 165.

The structure of the body wall in the genus *Arhythmorhynchus* presents numerous anomalies when compared with conditions found in other genera of *Acanthocephala*. The writer has made a study of some of these points, especially in the species *A. brevis*, the results of which follow. Lühe (1911: 47) has called attention to the peculiar distribution of the subcuticular nuclei in this genus and incidentally in a vague manner has referred to other differences between the anterior and posterior regions of the body. Figure 11 shows the shape, structure, and location of the subcuticular nuclei in a longitudinal section through the anterior region of a specimen of *A. brevis*. The entire subcuticula is a peculiar structure, presenting an appearance unparalleled in any other genus of *Acanthocephala*. In the anterior region of the body there may be easily recognized beneath the cuticula (*c*) a region in which small fibrillae run both longitudinally and radially (*sc1*). An intermediate more heavily granular zone (*sc2*) separates this region from the region of radial fibers (*sc3*) in which the subcuticular nuclei (*sn*) are contained. This last region, which Kaiser (1913, Plate 1, Fig. 1) in *Gigantorhynchus hirudinaceus* called the hypoderm, is bounded on its inner surface by a layer of circular muscular threads (*cmt*).

The longitudinal muscular layer in the anterior part of the body shows some most striking deviations from conditions usually found in the body musculature of *Acanthocephala*. The presence of large nuclei (*mn*) in, and of numerous finger-like fiber-bundles (*mf*) imbedded in an undifferentiated cytoplasmic envelop (*uc*) suggest a resemblance to

the nematode musculature. But this can be scarcely more than a suggestion since the orientation of the fibers is the opposite of that characteristic of the nematodes. Figure 11, a longitudinal section of *A. brevis*, shows these fiber-bundles in a position comparable to the view obtained in a cross section of a nematode. Though this agreement in fundamental structure of the muscle cells may indicate a relationship between the Acanthocephala and the Nematoda, yet the confusion in the arrangement of the fibers prevents ascribing to the argument any great phylogenetic importance.

Lühe in his characterization of the genus *Arhythmorhynchus* commented upon the slight development of the lacunar system of the subcuticula. In Figure 7, the writer has shown a portion of a tangential section through the subcuticula of *A. brevis*. A longitudinal canal (*lc*) is shown in its characteristic relationship with a circular canal (*cc*). In this species, at least, the canal system is well developed, though the extent and complexity of the subcuticular layer tend to make it inconspicuous.

In the posterior region of the body the body-wall presents its broadest departure in *A. brevis* from the conditions usually found in other genera. Here, as has been stated before, there are no subcuticular nuclei. The regions of the subcuticula (Fig. 8) agree in arrangement and general structure with those previously described for the same layer in the anterior part of the body. However, between the double row of circular muscle threads (*cmt*) and the body cavity is interposed a series of structures which are evidently modified continuations of the muscular system described for the anterior region of the body. In a longitudinal section, or in an optical section of a well prepared whole mount, this modified part has the appearance of a series of triangular elevations (*tr*) with the base of each triangle directed toward the layer of circular muscle threads. From the apex of each of these triangles is given off a fine membrane (*m*) which runs inward toward the longitudinal muscle sheath. Each of the triangular elevations is pierced by a canal (*ca*) about 0.025 mm. in diameter. These triangular ridges occupy only about one fourth the region between the circular muscular threads and the muscle sheath lining the body cavity. Most of the intervening space is open cavity intercepted at irregular intervals by very thin membranes (*ms*) of another series which do not take their origin or have their insertion in the triangular ridges. The open spaces between the membranes are in communication with the central body cavity as is especially shown by the presence within the chambers of large numbers of eggs and embryos (*e*) in various stages of formation.

Some of the muscles within the body cavity show a peculiar striation. Figure 6 represents a single fiber of one of the retractor muscles

greatly magnified. Regions of dark striations (*st*) alternate with bands of nonstriated structure, while the nucleus (*n*) is in a mass of undifferentiated cytoplasm at one side of the fiber.

ARHYTHMORHYNCHUS PUMILIROSTRIS NOV. SPEC.

Body of males and immature females with slight enlargement comprising about anterior fifth. Gravid females with posterior region of body enlarged, cylindrical, with irregularly distributed swellings. Females up to 30 mm. long. Maximum diameter fully gravid female, slightly posterior to middle of body, 1.5 mm.; diameter anterior region 0.9 mm. Neck naked, retractile, tapering toward proboscis; in size not sharply set off from body. Body for short distance behind neck set with small spines, 0.012 to 0.020 mm. long. Proboscis elongated, with conspicuous swelling near center; length 0.450 mm.; maximum breadth 0.180 mm.; breadth at tip 0.095 mm., at base 0.114 mm. Proboscis armed with sixteen longitudinal rows of hooks with fourteen or fifteen hooks in a row. Basal hooks nearly straight, thorn like, usually 0.035 mm. long. Heaviest hooks on ventral surface near middle of proboscis 0.030 mm. long. Hooks at tip slender, recurved, 0.030 to 0.035 mm. long. Cement glands in male extremely attenuated. Testes contiguous in region behind anterior swelling of body. Embryos 0.065 to 0.089 mm. long; 0.018 mm. wide; with three membranes, the middle one with an outpocketing at each pole.

Host *Botaurus lentigenosus* (Montag.), intestine. Type locality Washington, D. C. Cotypes in collection of Bureau of Animal Industry, Washington, D. C., Catalog No. 2076; and in the Helminthological Collection of the Department of Zoology, University of Illinois, Catalog No. 16, 166.

In its microscopic anatomy this species closely resembles that given for the preceding species. Figure 13, an optical section of *A. pumilirostris*, indicates the general distribution of the two types of subcuticular structure discussed under the morphology of *A. brevis*, while Figure 12 shows a single hard shelled embryo.

INTERRELATIONSHIPS OF THE SPECIES

Upon the basis of the characteristics of the proboscis hooks alone there is an indication of a natural division of this genus into two subgroups which make comparisons between species fairly certain even though the essential diagnostic facts for some species are not all known. One group consists of those species whose members possess a few extremely large hooks at the middle of the ventral surface of the proboscis; *A. frassoni* and *A. trichocephalus* fall within this group. In the second group the midventral hooks are but slightly larger than the

midlateral and middorsal hooks; to this belong *A. invaginabilis*, *A. brevis*, *A. uncinatus*, and *A. pumilirostris*. *A. brevis* and *A. pumilirostris* may be separated from *A. invaginabilis* upon the basis of the number of longitudinal rows of hooks upon the proboscis. For the last named species Lühe (1912:287) found twenty-two to twenty-four longitudinal rows of hooks. Eighteen are found in *A. brevis* and sixteen in *A. pumilirostris*. The separation of *A. uncinatus* is most sharply shown in a comparison of the size of the hooks. Kaiser (1893:15) found hooks upon the proboscis of *A. uncinatus* ranging from 0.056 to 0.120 mm. long while in *A. brevis* the writer has found the range in size of hooks to be from 0.030 to 0.047 mm., and in *A. pumilirostris* the longest hooks are 0.035 mm. long. *A. brevis* and *A. pumilirostris* are most readily separable one from the other by the fact that the former has the larger proboscis with eighteen longitudinal rows of hooks, while the latter has but sixteen longitudinal rows of hooks upon a much smaller proboscis.

At the end of his work on the Acanthocephala of the fresh waters of Germany, Lühe (1911:53) has considered a number of species which were insufficiently known to permit of classification in his system with certainty. Among these is a species *E. striatus* Gze. for which he has mentioned an apparent relationship with the genus *Corynosoma* through the shape of the embryos. Since this is the sole point where the present writer found the two species *A. brevis* and *A. pumilirostris* to differ from Lühe's description of the genus *Arhythmorhynchus* and since the figures and description of *E. striatus* agree also with that genus the writer can see no objection to including the species *striatus* within the genus *Arhythmorhynchus* as emended in the present paper.

KEY TO THE SPECIES OF ARHYTHMORHYNCHUS REPORTED FROM
NORTH AMERICA

- 1 (2) Hooks on mid-ventral surface of proboscis conspicuously larger than any others.....*A. trichocephalus* (R. Leuckart)
- 2 (1) Hooks on ventral surface of proboscis not conspicuously larger than on other surfaces.....3
- 3 (4) Longest hooks more than 0.100 mm....*A. uncinatus* (Kaiser)
- 4 (3) Longest hooks not more than 0.050 mm.....5
- 5 (6) Proboscis with sixteen longitudinal rows of hooks; embryos 0.065 to 0.089 mm. long and 0.018 mm. wide.....
.....*A. pumilirostris* Van Cleave
- 6 (5) Proboscis with eighteen longitudinal rows of hooks, embryos 0.076 to 0.100 mm. long, and 0.024 to 0.030 mm. wide.....
.....*A. brevis* Van Cleave

SUMMARY

Two new species of Acanthocephala from the intestine of *Botaurus lentiginosus* show close relationship to *Arhythmorhynchus frassoni*. They fail to agree with Lühe's definition of the genus *Arhythmorhynchus* in: (1) shape of the body; (2) location of the testes; (3) shape of the membranes surrounding the hard-shelled embryos. The original characterization of the genus is emended to include these forms which possess every other essential characteristic of the genus.

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EXPLANATION OF PLATES

All figures drawn from permanent, stained, balsam mounts with the aid of a camera lucida.

PLATE 1

- Figs. 1 to 3.—*Arhythmorhynchus brevis* nov. spec.
- Fig. 1.—Immature male, entire.
- Fig. 2.—Profile, dorsal surface, proboscis of mature male.
- Fig. 3.—Profile, ventral surface, same proboscis as in Figure 2.
- Figs. 4 and 5.—*Arhythmorhynchus pumilirostris* nov. spec.
- Fig. 4.—Male, entire.
- Fig. 5.—Profile, anterior end of body of same individual as shown in Figure 4.
- Fig. 6.—Proboscis hooks same magnification as Figures 2 and 3 of *A. brevis*.
- Figs. 6 and 7.—*A. brevis*.
- Fig. 6.—Muscle fiber from one of the retractor muscles.
- Fig. 7.—Portion of tangential section through cuticula and subcuticula showing relations of longitudinal (*lc*) and circular (*cc*) canals.

PLATE 2

- Figs. 8 to 11.—*A. brevis*.
- Fig. 8.—Portion of body wall in posterior region. Sagittal section. For details see text.
- Fig. 9.—Spines from anterior part of body wall.
- Fig. 10.—Embryos from gravid female.
- Fig. 11.—Portion of body in anterior region. Sagittal section. For details see text.
- Figs. 12 to 14.—*A. pumilirostris*.
- Fig. 12.—Embryos from gravid female.
- Fig. 13.—Anterior end of body, optical section, showing relative differentiation and distribution of subcuticula.
- Fig. 14.—Spines from anterior part of body.

PLATE 1

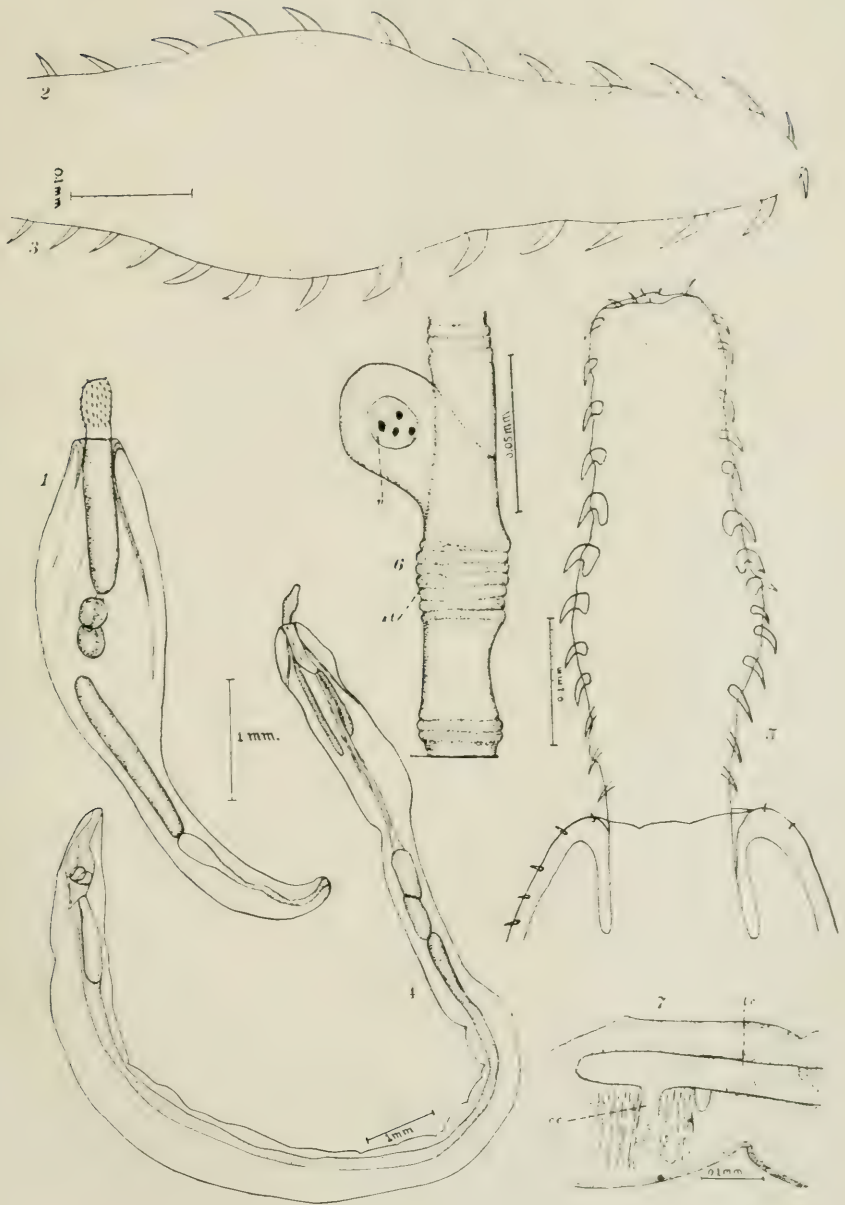
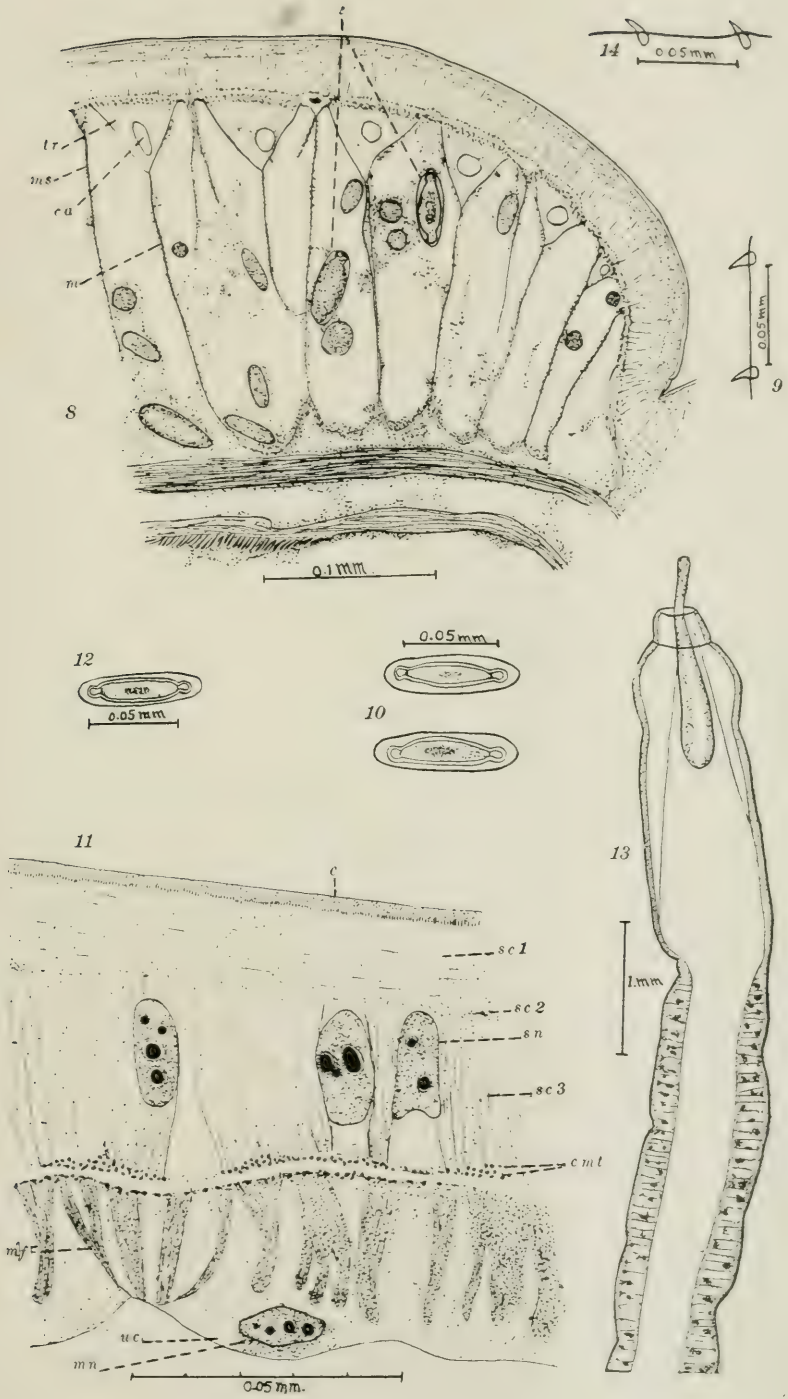


PLATE 2



SOME NOTES ON THE ENCYSTED LARVA OF THE LUNG DISTOME

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In a former article (1916) I reported on (1) the discovery of the intermediate hosts (crabs) of the lung distome in Japan; (2) species of the intermediate hosts in various districts of our country; (3) the frequency of occurrence of the encysted larvae (cysts) in various crabs; (4) morphology of the encysted larva; (5) the animals, experimentally fed with the cysts, etc. The report to be given in the following pages is a part of the results obtained by subsequent study on the cysts of the lung distome in crabs, especially *Eriocheir japonicus* (de Haan).

DISTRIBUTION AND MIGRATION OF CYSTS IN THE BODY OF AN INTERMEDIATE HOST

The encysted larvae are found in various parts of *E. japonicus*, namely, muscles, hypodermis, gills, liver and other organs; of these, muscles and gills are most abundantly infected. The absolute number of cysts is greater in the muscles than in the gills, but the relative number is inverse, because the volume of the muscles is much larger than that of the gills. The cysts in the gills are found only along a limited portion of blood vessels running longitudinally on the median line of their upper surfaces. In the muscles the cysts are found most frequently and most abundantly in the base of each appendage. Numerous cysts are often found in the muscles near the basipodite of each appendage, even in the cases in which a few or none of cysts are found in other parts of the musculature.

From the abundance of the cysts in gills and near their attachment, the basipodite of each leg, and from the system of blood circulation in the crab, I am inclined to believe that the encysted larvae have a tendency to migrate toward the gills from all parts of body by means of the blood circulation. It was experimentally proved that the cyst has the ability to migrate through the various tissues of the crab, although the rate of migration is very slow. On the other hand, the circulatory system of the crab is open, as the distal ends of the arteries open into the tissues of the body and thus all tissues are bathed in the blood. The venous system begins not with capillaries, as in a closed system, but with lacunae, lying irregularly among the tissues. The lacunar spaces in the tissues communicate with one another at first, and gradu-

ally form a canal system after union of several lacunae from different parts; ultimately these grow into the venous vessels which run toward the gills to purify the blood. The blood current among the tissues and in the vessels of the venous system surely facilitates the migration of the cysts toward the gills. If this supposition is right, it explains clearly why the cysts are found abundantly in the small blood vessels in the gills and in the muscle near the base of each appendage.

Thus the venous vessel is the most convenient course by which the cysts migrate toward the gills. For what purpose do the cysts migrate to the gills? Is there any necessity for the cysts to migrate to the gills? It is most favorable, I believe, for the cysts to migrate to the gills in order to facilitate further development of the encysted larvæ by getting into the final host. On the whole, there are two ways by which the cysts may be taken up by the final hosts—human beings or other animals as dog, cat, etc.—namely: (1) their consumption as a food in an uncooked crab; (2) being taken with food and drink infected with cysts liberated into water from the intermediate host. In the second method of infection it is necessary for the cysts to escape into the water from the infected crab. The gills are the most convenient point at which the cysts can escape easily into the water, because the organ is always being laved by water and the blood vessel containing the cysts is separated from the outside water only by the very thin membranous wall. Thus it is reasonable to think that the cysts in various parts of the intermediate host migrate through the tissues carried by the blood current in the venous vessels toward the gills from which they may be discharged into the water.

It is questionable in my mind whether the cysts in a crab (*E. japonicus*) escape into the water naturally and actively to secure an opportunity of being taken up by the final host. In Corea, R. Moriyasu, E. Arima, and M. Tanaka, proved experimentally the natural and active escape of cysts in the case of *E. japonicus*. In Formosa, K. Nakagawa obtained the same results as Moriyasu in the case of *P. obtusipes* (Stimpson). In Japan proper, R. Ando also proved experimentally that the result is quite the same in the case of *P. dehaanii* (White). All these writers made their experiments by approximately similar methods, namely, putting ten to thirty specimens of a crab which seemed to be infected with the cysts into cylindrical glass vessels with a little water. Renewing the water once or twice a day, they searched for cysts. In these examinations they all found the cysts more or less numerous, and hence concluded that the cysts escaped naturally and actively from the body of crab. In the case of *P. dehaanii* and *P. obtusipes*, it is possible that the cysts in the crab may escape into the water naturally and actively, because in these intermediate hosts the cysts are often found

attached to the outer surface of gills, as I reported in the former paper (1916).

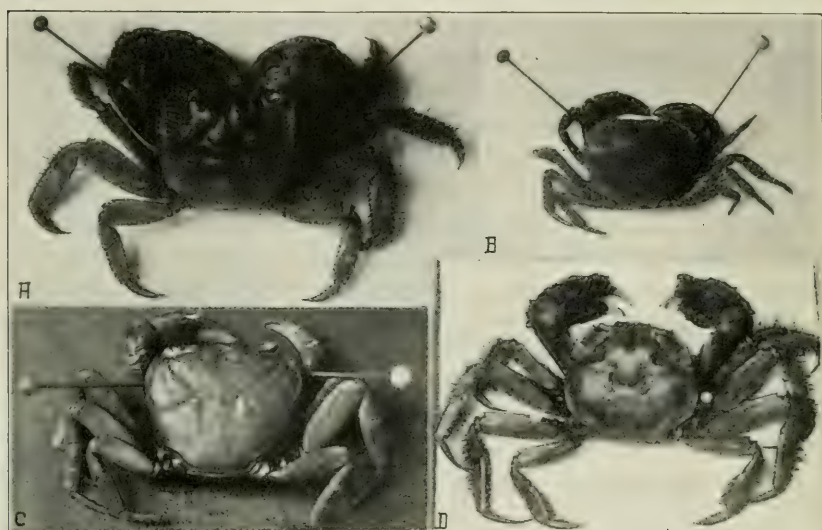
I have also made some experiments to prove the natural and active discharge of cysts from the crab. There were two sets of experiments: 1. I prepared a glass aquarium 75 cm. long, 27 cm. wide and 29 cm. deep, provided with three small exits on the bottom, the upper side being open and covered by metal gauze when necessary. Twenty crabs or more of moderate size were put into the aquarium, and water was permitted to flow in by a pipe and out through three exits which were closed by two or three sheets of gauze in order to prevent the escape of the cysts. I examined the sediment on the bottom occasionally for cysts and have often found them among materials there. 2. I put one or two specimens of crab into a cylindrical glass vessel 18 cm. in diameter and 15 cm. deep, pouring in water to a depth of 3 cm. or more. I prepared five such vessels, and renewed the water once or twice a day. This set of experiments was continued three months, having been started October 4 last year. In this long-continued experiment, only one cyst came under my observation. In these experiments I used the crabs *E. japonicus* (de Haan) from Tomioka, *Tokushima Prefecture*, which were abundantly and frequently infected with the cysts of the lung distome.

In the first set of experiments I frequently found numerous cysts among other sediment. I have occasionally found dead crabs in the aquarium and pieces of legs and other parts of the body were always present in the material on the bottom of the vessel. It is reasonable to think that the cysts in the crab are easily discharged from the body when the crab is dead or any part of body is accidentally injured. Hence from observation of the above facts I believe that these free cysts in the aquarium were unnaturally and passively discharged from the body by occasion of death or some injury.

When the crabs used in the second set of experiments were dead the substitute crabs were usually transported from the aquarium of the first set. It is even possible that the one cyst which I found in the second was not naturally and actively discharged from body of crab itself, but was carried attached to some part of the body from the aquarium in which I had proved the presence of cysts as stated above. The thickly haired forceps of the crab may be a good carrier of cysts, adhering to it even in case the crab had been carefully washed to remove attached particles.

In considering the facts observed in the above two sets of experiments one may say that if the cysts in the first aquarium had been naturally and actively discharged from the crabs I should have found more numerous cysts in the vessels of the second series than were found actually. But in reality, there was only one cyst in the vessels

of the first set during a long time. Thus I conclude from my own experiments that cysts in the intermediate host (*E. japonicus*) are not naturally and actively discharged from the body, but are often expelled unnaturally and passively by death of the crabs or some injury. In nature there are many occasions favorable for cysts escaping from crabs unnaturally and passively, namely, death of the crabs, frequent injuries by the fierce quarrels of the warlike crabs, breaking legs in slight disturbances, and accidental injuries in the period of moulting, etc.



Japanese River Crabs which serve as intermediate hosts for *Paragonimus westermanii*. A. *Sesarma dehaani* M. Edwards. B. *Potamon dehaanii* (White). C. *Potamon obtusipes* (Stimpson). D. *Eriocheir japonicus* (de Haan). Photographs by Mr. Koyama.

LONGEVITY OF CYST IN WATER

For studying the transfer of this cyst to a final host, it is most important to know how many days the cyst can be kept alive naturally in water. I have made the following experiments to determine this matter: To keep the cysts in water in a state as similar to natural conditions as possible, I prepared a small glass aquarium of 30 cm. long, 20 cm. wide and 17 cm. deep with the bottom provided with one small exit. Water was constantly pouring into it by the inflow pipe and flowing out through the exit on the bottom, so the water in the aquarium was always moving and being renewed as in a running stream. For convenience in examining perfectly changes in the cysts and counting accurately the number of cysts dead or alive, I used as a case for holding them a glass tube opening at both ends covered by one or two

sheets of gauze and filter paper to prevent the cysts escaping from the tube but to permit the water to flow in and out though not freely.

(A) I put forty-two cysts from the gills of *E. japonicus* in a tube whose ends were closed by gauze. The tube was placed in the aquarium October 30 and taken out for examination November 12, having been in water thirteen days.

(B) Twenty-five cysts from the gills of the same species of crab were put in a tube, one end of which was closed by layers of gauze and two layers of filter paper and the other end by two layers of gauze and one layer of filter paper. The tube was kept in the aquarium from November 12 to 27, an interval of fifteen days.

(C) Twenty-five cysts from the gills and muscles of the same species of crab were put in a tube whose ends were closed by two layers of gauze and two layers of filter paper. The tube was left in the aquarium from November 12 to December 10, or twenty-eight days.

(D) November 15 I removed the cysts with surrounding tissues of the host from the gills and muscles of a specimen of *E. japonicus* that had died November 12. Twenty of these cysts were put in a tube whose ends were closed as in Case C. The tube was immersed in the aquarium during twenty-five days from November 15 to December 10.

The results of these experiments are listed as follows:

Case	Total Number	Living One	Dead One	Cyst Only	Percentage	Days
A	26	10 (in cyst) 4 (outside)	4 (in cyst) 1 (outside)	4	53.8	13
B	25	4 (in cyst) 1 (outside)	5 (in cyst) 1 (outside)	14	20.0	15
C	25	(All were dead and decomposed)			28
D	20	2	2	14	10.0	25

In Case A twenty-six out of forty-two cysts were found in the tube and the remaining were lost. The loss may be perhaps due to having closed both ends of the tube with gauze only. To avoid this defect in Cases B, C and D, I had used both gauze and filter paper for closing the tube ends, the latter being placed inside of the former.

Cysts containing living larva were not all perfect, some of them being slightly broken and the others so widely broken that the larva was creeping out. I found there were living worms also in various stages. Some of them were actively moving with the light red pigment in the body as observed in fresh larvae, others moved slowly, and some others appeared dead, having no apparent motion. In the last group the light red pigment was greatly reduced or entirely absent. Various gradations of morphological change and putrefaction were observed in

dead worms. In almost all the cysts, whether the worm was alive or dead, swarmed an immense number of flagellata of various species.

From my experiments above it is evident that the encysted larva may be kept alive relatively long in water. If a larger tube be used instead of a small one as in my experiments and both ends of the tube be closed by other suitable materials which make the circulation of water in the tube as perfect as possible under the conditions that retain the cysts, putrefaction of the cysts and their surrounding host tissues would be delayed and consequently the cysts would remain alive for longer time. Therefore we may conclude that cysts in water remain alive at least for thirty days under natural conditions.

From my other experiments it is known that cysts in the crab may be kept alive for a week in the winter season, the gills and other inner parts of the crab being exposed to an air by taking off the carapace.

METHOD OF INFECTION

There are two ways in which the human host may be infected with encysted larvae from the crab: (1) by taking as food an uncooked crab infected with living cysts; (2) by taking with food and drink living cysts discharged from the crabs. Which of these two ways of infection is common may be quite different in various districts of the country, varying according to the species of intermediate host and to the custom of people in the district. One intermediate host, *E. japonicus*, is edible and used as food in all districts of Japan, but it is generally eaten cooked—boiled, roasted or fried—and is rarely used uncooked. Another crab, *P. dehaanii*, is also edible and eaten cooked or uncooked in general. In some districts it is customary to use it uncooked in certain season of year. People in these districts are easily and commonly infected by eating uncooked crabs and a large percentage of those people are found to be afflicted with lung distomiasis. *S. dehaani* is not taken as food and human infection will be brought about by the second method in the districts where *S. dehaani* happens to be the only intermediate host present.

For prophylaxis in the disease caused by the lung distome the following are necessary conditions: Not partaking of uncooked crabs and other foods washed in water in an infected district. Not drinking unboiled water in such district.

REFERENCE CITED

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CYLINDROTAENIA AMERICANA NOV. SPEC. FROM THE
CRICKET FROG *

MINNA E. JEWELL

In the fall of 1914, while looking for parasites, I found a cestode in the intestine of a cricket frog, *Acris gryllis*. Further collections were made and the repeated occurrence of the tapeworm showed that it was not merely incidental but a regular parasite in the host mentioned. This discovery was particularly interesting because of the rarity of cestodes, either species or individuals, in amphibians. So far as I have been able to ascertain, only five cestodes have yet been described from amphibians, and of these only two, *Taenia dispar* Goeze (1782), and *Taenia pulchella* Leidy (1851), are from Anura. No cestodes have ever been reported from a member of the genus *Acris*. For these reasons it was considered worth while to make a morphological and systematic study of this new form, the results of which are presented in the following paper.

I wish to express my thanks to Prof. Henry B. Ward for the use of his library and of materials from his private collections and for many helpful suggestions.

Aside from some fifty specimens I obtained from cricket frogs collected from a drainage ditch north of Urbana, Ill., specimens were also examined from *Rana pipiens* collected by W. W. Cort at Douglas Lake, Mich., and by R. G. Hall from Crystal Lake, Urbana; from *Rana virescens* collected by H. W. Duncanson near Peru, Neb., and from *Bufo lentiginosus*, locality unknown. Much of this material had been identified as "*Taenia dispar*" on the basis of its general form and of its host, but comparison of these specimens with specimens of *Taenia dispar* sent from Neuchâtel, Switzerland, by Dr. Otto Fuhrmann, showed conclusively that the American worms are a distinct species.

Taenia dispar was originally reported by Goeze from toads and frogs in Germany. He described it as being 6 inches long, cylindrical, of greatest diameter at the anterior end and diminishing gradually to a thread-like posterior end. The name "*dispar*" was suggested by this unusual shape. The color is white except at the posterior end, where it is brownish. Proglottids are distinct only near the posterior end in which region they are filled with numerous brown bodies. All of the proglottids are enclosed in a thin transparent membrane, which is

* Contributions from the Zoological Laboratory of the University of Illinois, under the direction of Henry B. Ward, No. 67.

clearly visible between the proglottids at the posterior end. Observations were made on living material placed in water and the great activity of the worm noted.

O. Schmidt (1855) studied some eighty or ninety specimens obtained from *Rana temporaria*. He pictures a worm in which the neck is pronounced, being about one half the diameter of the scolex and two fifths the diameter of the body where the testes are at their fullest development. Failing to find the female organs he apparently mistook the testes for ovaries, and while he gives us a description and figure of what is unmistakably an oval cirrus pouch, he fails to recognize it as such, but considers it a part of the female reproductive system. He describes in detail the development of the embryo and subsequent formation of capsules, each surrounding three embryos. Of these capsules he found nineteen to twenty-five in each proglottid, first arranged in the form of a circle, but later becoming scattered irregularly through the proglottid.

Fuhrmann (1895) summarizes the contributions of previous workers and adds a careful and detailed description of his own, a summary of which follows:

Taenia dispar is characterized by its cylindrical form and by the fact that its diameter is greatest at the anterior end and diminishes gradually toward the posterior end. The scolex is unarmed and is not separated from the body by a neck. The pores are lateral and the cirrus and vagina pass dorsal to the longitudinal excretory vessels and main nerve trunk (Textfig. A). The testes are dorsal, two in number, and measure 0.108 by 0.045 mm. The cirrus sac is a strongly muscular organ, having a length of 0.27 mm. and a diameter of 0.026 mm. It terminated in a retractor which extends to the muscle layer on the opposite side of the proglottid.

The female genital organs occupy the ventral part of the proglottid. The ovary is spherical, 0.081 mm. in diameter, surrounded by a delicate membrane and filled with forty to ninety cells 0.014 mm. in diameter. The vitelline gland is also spherical, but its cells are much smaller than those of the ovary. No shell gland was observed. The uterus first appears as a mass of dark cells between the ovary and testes. At its fullest development it is a large horseshoe-shaped organ, the dorsal part of which crowds the remnants of the testes against the dorsal muscles. The uterine wall soon degenerates and the eggs receive their second and then their third membranes from the parenchyma. The parenchyma now becomes concentrated about groups of three or sometimes four eggs, enclosing them in a parenchymatous capsule. These egg capsules, thirteen to thirty in number, become scattered irregularly through the proglottid.

It is noteworthy that there are marked discrepancies between the figures and descriptions of *Taenia dispar*, contributed by Goeze and Fuhrmann on the one hand, and O. Schmidt on the other. While the circular arrangement of the eggs described by Schmidt and the horseshoe-shaped arrangement described by Fuhrmann might readily be accounted for as differences in observation, there are more important differences which cannot be so readily explained. Whereas Goeze

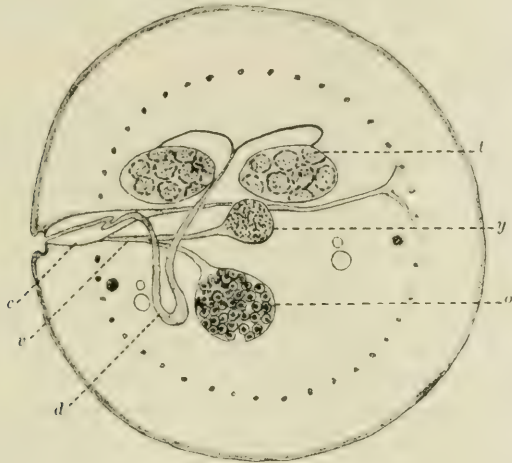


Figure A

Cross section of a mature proglottid of *Taenia dispar*. (After Fuhrmann, 1895); *t*, testes; *c*, cirrus pouch; *d*, vas deferens; *o*, ovary; *u*, uterus; *v*, vagina; *y*, vitellaria.

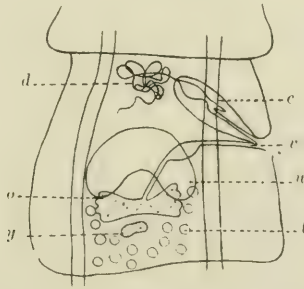


Figure B

Mature proglottid of *Paruterina angustata*, showing the arrangement of organs characteristic of the sub-family Paruterinae. (After Fuhrmann, 1906.)

and Fuhrmann both picture and describe *Taenia dispar* as neckless, having its greatest diameter at the anterior end and diminishing gradually toward the posterior end, Schmidt, as noted above, gives a picture of a worm with a pronounced neck and with its greatest diameter near the posterior end. Further, Fuhrmann describes the cirrus sac as being almost ten times as long as broad, while Schmidt pictures an oval

cirrus sac not more than twice as long as broad. These discrepancies would suggest the possibility that the form worked upon by Schmidt was not *Taenia dispar*, and that the number of taenian species found in amphibians is greater than has heretofore been supposed.

Weinland (1858) attempted to put this form in a new genus, *Proteocephalus*, a position which subsequent workers have shown to be untenable. Lühe (1899) proposed for *Taenia dispar* the generic name *Nematotaenia*, suggested by the cylindrical form and unsegmented appearance of the body. Ransom (1900) gives the following diagnosis for the genus *Nematotaenia*:

Paruterinae; scolex unarmed without rostellum. Segmentation of strobila distinct only at the posterior end. Strobila circular in cross section. Genital pores alternate; genital canals pass dorsal to the longitudinal excretory vessels and nerve. Uterus horseshoe shaped, disappears early. Eggs through the action of numerous parauterine organs become inclosed in egg capsules, three or four in each capsule. Adults in amphibia. Type species *Taenia dispar* Goeze 1782.

Stiles and Hassall (1912) record the following as hosts of *Taenia dispar*: *Bufo americanus*, *Menobanchus maculatus*, *Bufo vulgaris*, *Rana pipiens*, *Rana temporaria*, *Ascolobates mauritanicus*, *Bufo cinereus*, *Bufo fuscus*, *Bufo lentiginosus*, *Hyla arborea*, *Necturus maculatus*, *Pleobetes fuscus*, *Platydictylus guttatus*, *Rana halecina*, *Salamandra atra*, *Salamandra maculata*. It is likely that in some of the above cases, the worm found was not *Taenia dispar*, but the species under consideration in this paper or some form much like it.

Taenia pulchella is known only from the rather meager description given by Leidy (1851), which runs as follows:

White, without admixture of any other color, variable, usually broadest anteriorly. Head quadrilateral, subclavate, obtusely rounded, broader than neck. Acetabula circular, cup shaped, lateral and opposite, sessile protractile. Neck very long, cylindroid. Articuli containing several colorless globules; anteriorly subglobular or transversely oval; posteriorly moniliform, longitudinally oval, or cylindroid and centrally incassate. Entire length, 50.8 to 238.6 mm. Scolex, diameter, 0.34 mm. Acetabula, 0.127 to 0.153 mm. Anterior proglottids, length, 0.34 mm.; diameter 0.254 to 0.53 mm. Ripe proglottids, length, 0.53 to 0.57 mm.; diameter, 0.19 to 0.34 mm. Host, *Bufo americanus*.

Closely resembles *Taenia dispar* Goeze, found in *Bufo viridis*, etc., but it is relatively longer and narrower and is never colored.

Morphology of the New Species.—In making and tabulating measurements, the worms from *Acris gryllis* were found to fluctuate about a different mode from those from *Rana pipiens*, being usually smaller; however, since every gradation in size has been found and the larger worms from *Acris gryllis* are larger than the smaller ones from *Rana pipiens*, and since also it has been observed in *Acris gryllis* that in cases of heavy infection mature worms are much smaller than those found in lightly infected hosts, sometimes not more than one half the diameter, the author feels no hesitation in saying that only one species is concerned.

Adult worms bearing ripe proglottids, from *Acris gryllis*, vary in length from 25 to 40 mm. when in a state of moderate extension. Young worms 1.5 mm. in length were repeatedly found in the intestine. The worms from *Rana pipiens* were not observed alive, but from preserved materials their length may be estimated to have reached a maximum of 80 mm.

The most characteristic feature of the worm, noted upon a superficial examination, is its cylindrical form. The color is glistening white throughout the entire length. The scolex is spherical, 160 to 200 μ in diameter in the region of the suckers, which have a diameter of from two fifths to one half that of the scolex. Thus in one scolex having a diameter of 180, the suckers are 97 μ . The neck is long and has a diameter of 130 to 150 μ . In a typical specimen from *Acris gryllis* 37 to 40 mm. in length, the neck has a diameter of 134 μ . The first appearance of the reproductive system is as a dark streak down the center of the worm about 5 mm. behind the head. Here the diameter is still 134 μ .

Soon the line of undifferentiated cells becomes broken into triangles, having their bases directed laterad and their species alternating with each other in the median line. Six mm. behind the head the differentiation of the testes becomes apparent. In this region the proglottids have a length of 9 μ and a diameter of 162 μ . Eleven mm. behind the head the proglottids are mature and the first eggs are passing into the uterus. Here the proglottids measure 20 by 157 μ . The greatest diameter of the worm is found where the uterus has reached its fullest development and the para-uterine organ is forming, about 22 mm. behind the head. In this region the proglottids are 40 to 45 μ long and 180 to 200 μ in diameter. When the worm is contracted the diameter may be 350 μ .

Soon after, about 24 mm. from the head, the proglottids begin to elongate rapidly and indications of external segmentation appear. They now have a length of 54 and a diameter of 135 μ . From 27 to 36 mm. behind the head the segmentation is very distinct. The proglottids measure 82 by 108 μ and break off easily. The last few proglottids of a strobila and the detached proglottids frequently have a length much exceeding their diameter, 146 by 72 μ to 178 by 74 μ . In specimens from *Rana pipiens* the proglottids attain a maximum diameter of 270 μ and ripe proglottids a length of 340 μ and diameter of 250 μ . Detached ripe proglottids have been found singly and in groups of from two to five in the cloaca of the host.

In living material the parenchymatous para-uterine organs which contain the oncospheres appear as two transparent spherical bodies in the center of each proglottid.

The cuticula is from 3 to 4 μ thick, and composed of three layers, the central one of which is thinnest. Beneath the cuticula is the usual

basement membrane and parenchyma. The subcutaneous muscles are weakly developed, the longitudinal muscles are pronounced, dorso-ventral muscles appear to be entirely wanting.

The ventral excretory canals vary in diameter from 3.5 to 12μ , usually from 5 to 7μ in parts anterior to the appearance of external segmentation. The dorsal canals vary in diameter between 1 and 4.5μ . They are but little smaller than the ventral canals in the region of the scolex, but are insignificant throughout the remainder of their length. The usual median excretory bladder is clearly visible at the posterior end of young specimens.

All of the organs of the reproductive system, with the exception of parts of the cirrus and vagina, are confined to the medullary region of the proglottid. The genital pores are lateral and alternate somewhat irregularly, though with a marked tendency toward regularity. Thus in one instance twenty-four pores alternate regularly, then two are on the right margin and the next two on the left; then four alternate regularly, two are at the right, five alternate regularly and two more are at the right, twelve alternate regularly and two are at the left, etc. More than two pores have never been observed to occur successively on the same side.

The cirrus and vagina pass dorsal to the main excretory canals and nerve trunk. The male organs occupy the dorsal part and the female organs the ventral part of the proglottid (Fig. 7). The single testis is situated dorsally on the aporal side of the proglottid. It varies from 26 to 34μ in diameter, being usually about 29μ at its greatest development, and is spherical except when anteroposteriorly compressed by the contraction of the worm. From it the vas deferens leads with but few undulations directly to the cirrus. This latter organ is surrounded by a thick club-shaped cirrus pouch 36 to 44μ long and 13 to 17μ in diameter. The cirrus pouch, vas deferens, and female organs are enclosed in a delicate sheath.

The vagina opens from the genital cloaca posterior to the male orifice and follows the cirrus inward. Near the end of the cirrus sac the vagina begins to curve ventrad. It meets the duct from the vitelline gland and the very short oviduct about the level of the principal nerve trunks. The single spherical ovary lies in the ventral half of the medullary region. It has a diameter of from 24 to 34μ , and contains from eight to sixteen large, spherical, loosely arranged cells 9μ in diameter, surrounded by a membranous capsule. The vitelline gland lies dorsolateral to the ovary and in the median line. It is spherical, 18μ in diameter, and composed of large deeply staining cells. The vitelline duct passes laterad, meeting the oviduct in an enlargement at the point of formation of the uterine duct. No special muscular oötype has been observed. A mass of deeply staining cells dorsal to

the vitelline duct is the anlage of the uterus. After fertilization the distal end of the oviduct becomes dilated and filled with sperm and yolk cells through which the egg must pass before entering the uterus.

The ova, when mature, pass in rapid succession through the oötype and into the uterus so that the ovary and vitelline glands soon disappear entirely. The uterus, an oval sac, lies on the pore side of the proglottid with its long axis directed dorsiventrally. At its fullest development it attains a size of 40 by 24 μ . The eggs at the time they enter the uterus may be surrounded by a transparent membrane, though groups of ova and yolk cells around which no membrane has yet formed are frequently found in the uterus. The complete eggs have a mean diameter of 12 to 14 μ .

The parenchyma on the aporal side of the uterus now becomes arranged as a meshwork of heavy deeply staining strands running parallel to the long axis of the uterus. This is the beginning of the parenchymous structure which, following Fuhrmann, I shall term the *para-uterine organ* (Fig. 2).

The growth of the para-uterine organ is rapid, and it soon appears as two truncated cones, one dorsal and one ventral, their bases lying against the uterus, which has become much flattened, and their apices extending almost to the circular muscles on the opposite side (Fig. 3). The basal portion of the cones is composed of a meshwork of fine dorsiventrally directed fibers. The apical parts are surrounded by heavy deeply staining fibers, among which lie numerous dark nuclei.

Meanwhile the eggs have initiated cleavage and have developed their second membrane, a thick deeply staining capsule, while the uterus, which was pushed close against the eggs by the growth of the para-uterine organ, has broken down into a number of tertiary capsules surrounding the individual embryos.

With the rapid elongation of the proglottid (Figs. 4 and 5) the position of the cones is shifted so that their longitudinal axes correspond very nearly to the longitudinal axis of the worm. Their apices lie in the anterior end of the proglottid and their basal portions, in which are the embryos enclosed in their uterine capsules, occupy the posterior part of the proglottid. At the same time the apical portions of the cones acquire well-defined walls and become somewhat constricted from the basal portions, while the spongy fibers which have filled them disappear leaving them hollow. By the time the proglottids have become distinctly set off, the apical portions of the cones appear as two thick-walled hollow spheres 20 μ in diameter, lying one dorsal, the other ventral, in the anterior end of the proglottid, while the meshwork of lamellated fibers has largely disappeared from the interior of the basal portions of the cones. At this time the embryos have a diameter of 20 μ and have developed the six hooks characteristic of the tapeworm oncosphere.

The oncospheres now begin to migrate forward into the spherical capsules of the para-uterine organ, which grow rapidly to a diameter of 124 to 130 μ (Fig. 6). At the time of the separation of the proglottids those embryos which have not yet migrated into the para-uterine capsule are usually set free by the tearing open of the end of the proglottid, so that a detached proglottid, when found in the cloaca of the host, frequently contains not more than five to seven oncospheres (Fig. 9).

The development of the para-uterine organ just described bears many resemblances to that described for *Metroliasthes lucida* by Ransom (1900). The chief differences are in the relative size and duration of the uterus and the number of para-uterine organs formed. In the form under discussion, as noted above, the uterus is relatively small and breaks down into membranes surrounding the embryos long before the development of the oncospheres is complete or the para-uterine capsule is ready to receive them. In *Metroliasthes lucida*, quoting Ransom, "at the height of its development the uterus occupies almost the whole of the inner parenchyma back to the genital pore and bulges out the proglottid wall dorsally and ventrally," and the uterus does not degenerate until the six-hooked embryos have taken up their final position in the para-uterine capsule. As to the number of para-uterine capsules formed, while in the form under discussion there are two, *Metroliasthes lucida*, although possessing a two-lobed ovary, has but one.

Fuhrmann (1906) has given in less detail the development of the para-uterine organ in *Paruterina angustata* and *Culcitella rapaciola*, (1908a) of *Anonchotaenia globata* and (1909) of *Biuterina clavulus*. Cholodkovsky (1906) has given a brief account of the formation of the para-uterine organ in *Rhabdometra tomica*. All of these forms resemble *Metroliasthes lucida* in that the uterus persists until the oncospheres have passed into the single para-uterine organ. *Taenia dispar*, on the other hand, resembles this form in that the uterus breaks down early, but far exceeds it in the number of para-uterine organs, of which there are from thirteen to thirty.

While the form under discussion bears some likeness to *Taenia pulchella* Leidy 1851, such as its occurrence in an anuran, its long neck, white color and cylindrical form, this similarity is far too generalized to establish identity. Since I have been unable to secure for comparison any of Leidy's material, which is reported to be no longer in existence, I must leave open the question of the possible identity of the two forms and treat this as a new species.

Fuhrmann (1908b) has revised the classification of the Cyclophylloids. Of his seven families it is the *Dilepinidae* with whose characters this worm agrees. The family is defined as follows: "Rostellum

usually armed, suckers unarmed, genital pores marginal, genital organs single or double in each proglottid." This family contains twenty-eight genera, which Fuhrmann has separated into three subfamilies on the basis of the character of the uterus.

The subfamily *Dilepinae* contains the genera in which the uterus is sac-shaped or has simple lobes. In most the uterus persists. The subfamily *Dipylidiinae* includes the genera in which the uterus breaks up into parenchymatous capsules which contain one or more oncospheres. The subfamily *Paruterinae* includes those genera in which a parenchymatous para-uterine organ is formed into which the embryos later penetrate. The enclosure of the embryos in the para-uterine capsule places the worm under consideration in this paper in the subfamily *Paruterinae*.

A comparison of the description of this form, given above, with the description of *Taenia dispar* given by Fuhrmann, reveals striking resemblances between the two (Fig. 7 and Textfig. A). Alike they are characterized by their cylindrical form and late differentiation of proglottids. The ovary and vitellaria are spherical and ventral, the vitellaria, however, being dorsal to the ovary. The testes are large, dorsal and of a definite and limited number, one in this form, two in *Taenia dispar*. The cirrus and vagina are dorsal to the longitudinal excretory canals, and the number of eggs produced in each proglottid is small; eight to twelve in the former, not more than ninety in the latter. They are further alike in that the uterus breaks down early before the para-uterine capsules have been formed, and in having more than one para-uterine organ.

Of the other six genera of the *Paruterinae*, five: namely, *Paruterina*, *Biuterina*, *Culcitella*, *Rhabdometra* and *Metroliaesthes*, are alike flattened dorsiventrally, the proglottids are distinct at the time of maturity or earlier, the female reproductive organs are anterior to the testes and the vitellaria posterior to the ovary. The testes are small, numerous (twenty to forty), and of an inconstant number, and occupy the posterior part of the proglottid (Textfig. B). The cirrus and vagina pass between the excretory canals. (In *Paruterina angustata*, Fuhrmann, the dorsal canal has not been observed. The genital ducts, however, pass dorsal to the ventral canal.) That the eggs are very numerous is suggested by the pictures, though no one has ever counted them. The uterus is relatively large and persists until the embryos pass into the single para-uterine organ.

Thus it is seen that the genera of Fuhrmann's subfamily *Paruterinae* fall into two distinct groups in one of which is *Nematotaenia*; in the other the five genera named above. The genus *Anonchotaenia* differs somewhat from either group. While the testes are small and numerous, they are dorsally situated, and the ovary, vitellaria and uterus are arranged laterally from the genital pore in the order named. How-

ever, this difference in the position of organs seems to be brought about by the shortness of the proglottid which would not admit the anteroposterior arrangement common in the other forms. Since, therefore, *Anonchotaenia* is similar in general characters and in the aspect of the mature proglottid, and since the development of the para-uterine organ and of the uterus, which persists until the embryos have passed into the para-uterine organ, resembles that of *Paruterina* and *Biuterina* much more closely than that of any other form, I consider that *Anonchotaenia* is rightly placed in the subfamily with *Paruterina*, *Biuterina*, *Culcitella*, *Rhabdometra* and *Metroliaesthes*.

For *Nematotaenia* and the species under consideration in this paper, because of the pronounced differences in the aspects of the mature proglottids, the early degeneration of the uteri and the late formation of the para-uterine capsules, of which there are more than one in each proglottid, it seems necessary to establish a new subfamily, *Cylindrotaenianae*: Cylindrical Dilepinidae having one or two dorsally placed testes, ovary and vitellaria ventral, vitellaria dorsal to ovary. Proglottids distinct at the posterior end only. The uterus breaks down early and the embryos are later enclosed in para-uterine capsules. *Taenia pulchella* Leidy would probably also belong to this subfamily.

On the other hand, notwithstanding their marked similarity in the respects noted above, *Taenia dispar* and this new worm show certain important differences. As to external characters it may be mentioned that whereas the former has its greatest diameter at the anterior end and diminishes gradually to the posterior end, the latter has its greatest diameter about midway of the strobila and narrows toward both ends.

Of greater importance is the difference in the male reproductive system. Whereas *Taenia dispar* has two symmetrically placed oval testes and the vas deferens forms a loop which passes ventrad as far as the excretory canals, this new worm has a single spherical testis situated lateral to the median line of the proglottid and a simple straight vas deferens. And whereas the cirrus sac of *Taenia dispar* is almost ten times as long as wide, that of the worm herein discussed is only two and one-half times as long as wide.

Nowhere are the differences more striking than in the development of the para-uterine organs (Figs. 5 and 12) and the aspect of the ripe proglottids (Figs. 9 and 11) for in place of the two large elaborate cone-shaped structures noted above, which are probably the most noticeable and characteristic structures of the worm, *Taenia dispar* has a varying number of small para-uterine organs in no wise characteristic; and in place of the two large, transparent, spherical para-uterine capsules found in the ripe proglottids of this form, the ripe proglottids of *Taenia dispar* have from thirteen to thirty small dark capsules scattered through the parenchyma.

From these considerations it becomes evident that this form does not belong to the genus *Nematotaenia*, which it most closely resembles of any of the genera yet established, and it is necessary to establish a new genus for its reception.

This generic description would be as follows:

Genus *Cylindrotaenia*. Scolex unarmed, without rostellum; reproductive organs single in each proglottid; pores lateral, alternating; vagina and cirrus dorsal to the excretory canals and main nerve trunk; testis one, dorsal; ovary and vitellaria ventral. Uterus breaks up into capsules surrounding the embryos which ultimately pass into two paruterine capsules.

Type species *Cylindrotaenia americana*. Characters given above. From small intestine of various Anura. Type specimens in the collections of Henry B. Ward and M. E. Jewell.

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EXPLANATION OF PLATE

<i>a</i> Apical portion of Para-uterine organ	<i>p</i> Para-uterine organ
<i>b</i> Basal portion of Para-uterine organ	<i>r</i> Receptaculum seminis uterinum
<i>c</i> Cirrus pouch	<i>s</i> Septum between proglottids
<i>e</i> Eggs	<i>t</i> Testis
<i>ec</i> Excretory canals	<i>u</i> Uterus
<i>em</i> Embryo	<i>v</i> Vagina
<i>m</i> Longitudinal muscles	<i>vd</i> Vas deferens
<i>n</i> Nerve	<i>vt</i> Vitellaria
<i>o</i> Ovary	

All figures from camera lucida tracings except 7 and 8 which are reconstructions.

Figures 1-10 *Cylindrotaenia americana*.

Fig. 1.—Scolex, $\times 37$.

Fig. 2.—Cross section of a proglottid with fully developed uterus and para-uterine organ forming, $\times 165$.

Fig. 3.—Cross section of a proglottid in the region of greatest diameter, $\times 160$.

Fig. 4.—Cross section of a proglottid at the beginning of external segmentation, $\times 160$.

Fig. 5.—Toto mount, lateral view of a somewhat later stage, $\times 175$.

Fig. 6.—Toto mount, lateral view of a proglottid near the end of the strobila, $\times 235$.

Fig. 7.—Cross section of a mature proglottid, $\times 165$.

Fig. 8.—Cross section of a somewhat later stage showing the formation of the uterus, $\times 165$.

Fig. 9.—Detached ripe proglottid, $\times 235$.

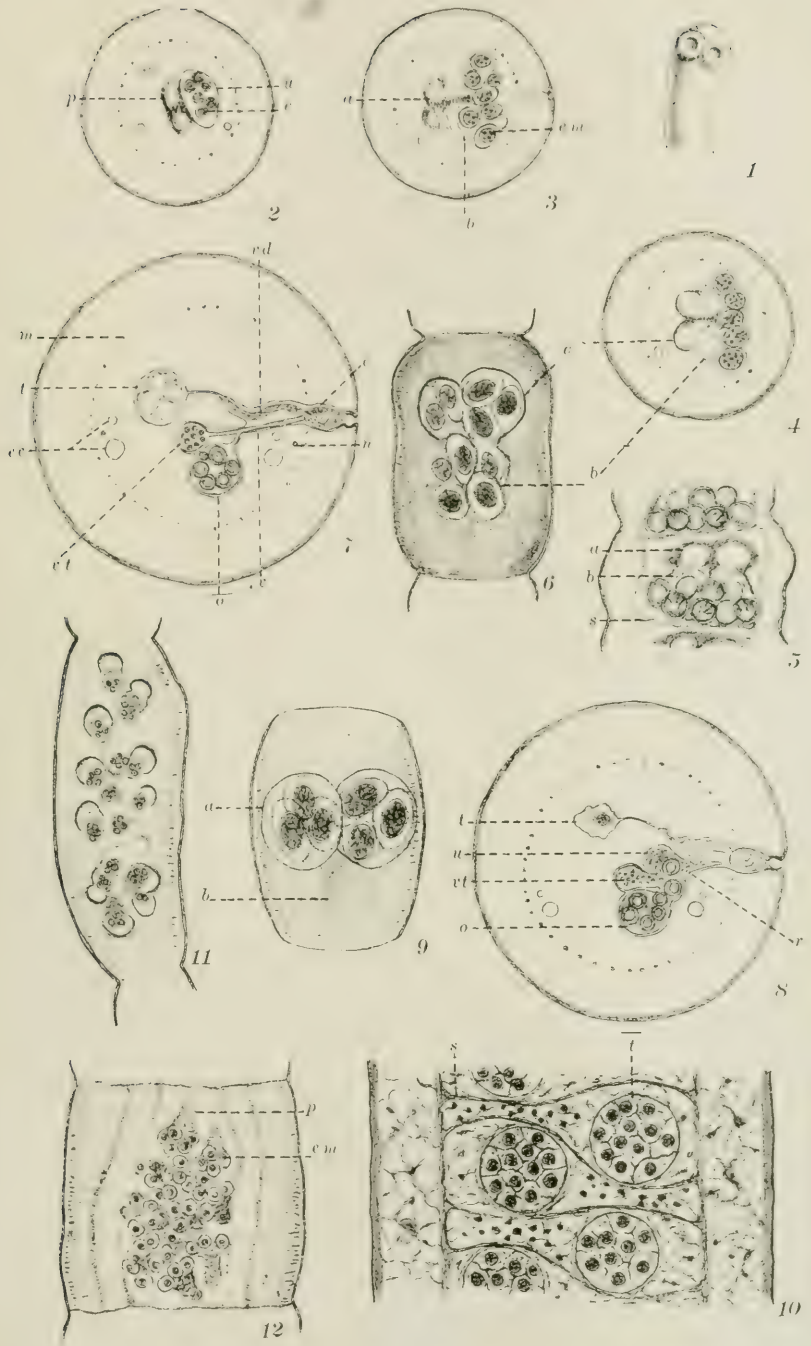
Fig. 10.—Frontal section of three proglottids in the region of the greatest development of the testes, $\times 325$.

Figs. 11 and 12.—*Taenia dispar*; from materials sent from Neuchatel, Switzerland.

Fig. 11.—Toto mount, ripe proglottid. Stage corresponding to Figure 9 in *Cylindrotaenia americana*, $\times 50$.

Fig. 12.—Toto mount, proglottid from near the end of the strobila showing para-uterine organs. Stage corresponds to Figure 5, $\times 70$.

PLATE



THE EFFECT OF TICK BITES ON MAN

D. McCaffrey

PRINCETON, B. C., CANADA

The local and constitutional effects which tick bites have on human beings is a subject which is still in the experimental stage, and I have been unable to find it discussed at any length in any of the textbooks. Osler merely mentions the fact. The only literature available is to be found in papers published in the scientific magazines.

I have had several cases which I attribute to the bites of ticks. These cases present two sets of symptoms, that is, local and constitutional. Where the local symptoms appear the tick has been forcibly removed, and the parts which it buries into its host left behind. The constitutional symptoms appear when the tick is allowed to remain on the host for some time. To illustrate the local symptoms I will describe two cases.

CASE 1.—M. M., aged 34. One night in May, 1913, on retiring, he felt an irritation along the right tibia. He rubbed the leg vigorously with the hand. Next morning the pruritus was very marked and a red spot appeared on the leg. The body of the tick was found on the floor. That day the leg began to swell and continued to do so until it was nearly twice its normal size. An abscess developed at the point where the tick's head was embedded. Hot poultices were applied for a week, then an incision made. The skin over the abscess was very tough. A creamy looking pus escaped. In about three days a hard dark colored mass came away. This was almost of a rubbery nature, and left an ulcer which extended nearly to the bone. I treated this as an ordinary ulcer for a week, but it showed no signs of healing. By that time the ulcer had hard indurated edges, giving it a "punched-out" appearance. There was a thin watery exudate coming from it. The only thing the patient complained about was the intense itching. In two months the ulcer was covered with skin. Each spring an ulcer has formed at the same place, which takes from two weeks to two months to heal. The leg itches almost constantly, at times becoming almost unbearable.

CASE 2.—Female, age 9, June, 1915. The tick was found on the mastoid bone. Her father removed it by force. Next morning the child complained of itching, and a red spot appeared. In two days the parts were much swollen and tender, with a raised spot where the head was imbedded. Applied hot poultices for four days and incised. The skin was very tough and very little pus escaped. The next day pus came from the ear as well as by the incision. On the third day after the incision was made, a dark, rubbery mass came away, leaving a "punched-out" ulcer. The ulcer has not healed as yet, Aug. 15, 1915, the pruritus being so severe that the child's hands have to be tied at night.

To illustrate the constitutional symptoms I will describe the only case I have had.

CASE 1.—D. W. Female, aged 11. May, 1915. Retired May 9, in ordinary health. When she got up the next morning her legs gave way and she fell. She walked about that day with no other symptom than falling, if she turned

or moved quickly. She could execute any movement if done slowly. The next day she could not walk and her arms were involved. Paralysis gradually extended until all the muscles were involved, leaving the child helpless. The pupils gradually dilated and lost their power of reacting to light. The tendons were at first exaggerated but later became lost. For the first four days the patient was very excitable. The muscles twitched so as to give her choreic movements; afterward she became somewhat duller but still retained most of her faculties. The involuntary muscles were the next to become affected, so that there was incontinence of the urine and feces. The breathing which was at first rapid became "choky," there being a peculiar rattling sound at each effort to breathe. She complained of a lump in her throat. If given liquids they returned by the nose. Her speech was affected so that she could hardly articulate. The tongue became swollen. The heart became very rapid, being above 120 per minute. The temperature at first rose 1 degree then dropped to 3 degrees below normal. Urine analysis negative. Sensory nerves normal. On the seventh day after symptoms appeared I removed a tick from near the crown of the head. Recovery was very rapid, so that on the third day she was able to walk up the street. In this case I had practically given up all hope of recovery. When the tick was removed I stopped all medicine and treatment. The child made a complete recovery.

I was fortunate enough to have the tick in this case identified as *Dermacentor venustus*. Whether it is *Dermacentor venustus* which causes the local effects or *Dermacentor albipictus*, which is also quite plentiful on horses in this district, I am unable to state. Princeton has an altitude of 2,000 feet and ticks are most abundant and active during the spring months and early summer; possibly they may be found higher up in the mountains at a later date. The case of "tick paralysis" I have just described is the first one of its kind that I have seen in the Princeton district. A number of cases have been reported from other parts of British Columbia, the nearest being in the Similkameen Valley some miles distant.

Dr. S. Hadwen examined the tick which was removed from the case of "tick paralysis," and determined it as *Dermacentor venustus*, a half-gorged female. He tells me that he has never seen any harmful effects from the bites of *D. albipictus* in animals, but that the local after-effects from the bites of *D. venustus* are often severe. According to Hadwen, the constitutional effects in animals following the prolonged attachment of *D. venustus* are identical to those I have just described.

SOCIETY PROCEEDINGS

THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON

The twenty-eighth regular meeting of the society was held at the residence of Mr. Crawley Dec. 10, 1915, Mr. Crawley acting as host and Mr. Chambers as chairman. Dr. Cobb presented the following:

Notes on New Genera and Species of Nematodes.—Note 1.—*Antarctic Nematodes*

The free-living marine nematodes of the Mawson Antarctic Expedition represent twelve species, nine of them new (one new genus), and three species previously described in my report on the free-living nematodes of the Shackleton Expedition. This raises the number of known marine species of Antarctic free-living nematodes to thirty-four, representing eighteen genera, only three of which are new. Considering the small number and the meagerness of the Antarctic collections, these results indicate that Antarctic species of marine free-living nematodes are very numerous and belong to very widely different genera, and for the most part to genera found in warmer seas.

NOTE 2.—*Renette of Cephalobus*

I find that in some species of *Cephalobus*, and probably in the majority, the excretory or renette duct is bifurcated and passes along the lateral fields to near the posterior end of the body. This structure thus parallels that found in many species of *Rhabditis*, and as a considerable number of parasitic forms have either rhabditiform larvae, or rhabditiform free-living generations, the possibility is suggested that *Cephalobus*, or rather some species of it, may be free-living forms connected with parasites. Examination of a large number of marine free-living nemas (a contracted term proposed here for the word nematodes) has strongly impressed me with the possibility that some of these species will in the end prove to be free-living forms of parasites of fishes, marine birds, cetaceans, etc.

NOTE 3.—*A New Form of Nematode Hermaphroditism*

I have a new nematode species that is extremely interesting in the form of its hermaphroditism. The individuals have the form of females, with double sex organs, one of normal size and functioning as an ovary, the other exceedingly small, and appearing to function as a testis.

NOTE 4.—*Subdivisions of Mononchus*

I find the free-living nematode genus *Mononchus* Bastian, 1866, to be divisible into five very natural divisions, of which the first three form a group considerably differentiated from the two others which may later be raised to the rank of genera.

1. *Mononchus* typical subg.—Pharynx twice to thrice as long as wide; onchus massive, midway or farther forward, unopposed by denticles; pharyngeal walls smooth or transversely striated; males of six species known; ovaries, two, reflexed. Type species *M. truncatus* Bast. Consisting of such species as *M. brachyuris* Bütsch.; *M. parvus* de Man; *M. rex* Cobb; *M. fovearum* Duj.; *pillatus* Bast.; *M. intermedius* Cobb; *M. major* Cobb; *M. gerlachei* de Man;

M. macrostoma Bast.; *M. longicaudatus* Cobb; *M. tunbridgensis* Bast.; *M. dadayi* Micol.

2. *Prionchulus* subg. nov.—Pharynx about twice as long as wide; onchus massive, midway or farther forward, opposed by numerous denticles arranged along a longitudinal pharyngeal rib; males of one species known; ovaries, two, reflexed. Type species *Pr. muscorum* (Duj.). Consisting of such species as *Pr. muscorum* (Duj.) and *Pr. spectabilis* (Ditlevsen).

3. *Mylonchulus* subg. nov.—Pharynx goblet-shaped; onchus more or less arcuate, massive, midway or farther forward, opposed by numerous denticles arranged in transverse rows on two rasp-like areas; males unknown; ovaries, two, reflexed. Type species *My. minor* Cobb. Consisting of such species as *My. minor* Cobb and *My. obtusicaudatus* (Daday).

4. *Iotonchus* subg. nov. (gen. nov.?).—Dorsal onchus and all others usually basal, relatively small; large species with large, elongated pharynx, having three longitudinal ribs; tail rather long and slender; males of two species known; ovaries, one or two, reflexed. Type species *I. gymmolaimus* Cobb. Consisting of such species as *I. digiturus* Cobb; *I. bathybius* (Micol.); *I. studei* (Steiner); *I. tridentatus* (de Man).

5. *Anatonchus*, subg. nov.—Onchi retrorse, midway in pharynx or sub-basal; large species with roomy elongated pharynx; tail long and usually becoming cylindroid; female organs double; males of most of the species known. Type species *A. tridentatus* (de Man). Includes *A. dolichurus* (Ditlevsen).

I have manuscript descriptions of several new mononchs from various parts of the world, all readily referable to one or another of these divisions.

NOTE 5.—Finder Slides

In an article in the Transactions of the American Microscopical Society (34:1-89) I have suggested the advisability of using co-ordinate numbers, preferably probably minus co-ordinates, dating from the upper right corner of the slide as the origin, or zero point. The slide I am exhibiting is of this kind, and presents the peculiarity that it does not have to be constantly removed and replaced when in use, thus effecting a material saving in time and energy. It consists of a series of coordinates arranged in a small holder adapted to receive and clamp the microscope slide upon and in register with the finder. Light from the microscope mirror passes through the finder and the microscope slide.

In other words, the finder slide is ruled into millimeter squares, each square containing two numbers indicating the actual distance of the square from the right-hand edge of the slide and from the top of the slide, respectively. Under the microscope the normal inversion makes these numbers appear to read from the left-hand side and from the bottom of the slide. The slide which is being studied fits over the finder and is held by two small fixed clamps. By focusing down at any point the two indicative numbers for the corresponding square may be found and noted. The slide is made by photographing a ruled and numbered sheet with such a reduction as will make the photographic squares one millimeter square.

Dr. Stiles presented a note in regard to the sanitary index of three Southern communities, A, B and C. In two of these communities, A and C, the authorities in charge had preached what they regarded as feasible, but comparatively low, standards of sanitation, including the advocacy of the unsheltered or so-called "umbrella privy." In the third community, B, the authorities had taken the position that it was not advisable to advocate something that would have to be combated subsequently, and in consequence high, even if temporarily unattainable, standards had been advocated. After the lapse of a year, the sanitary index of the three communities was again taken and compared with the index for the period of the sanitary campaign of a year before. It was

found that the sanitary index for the two communities A and C had fallen in a year from 28.1 to 24.2 for A, and from 34.6 to 29.4 for C, while the sanitary index had risen for community B from 31.5 to 45. The sanitary campaign in communities A and C was of the revival type with much attendant publicity; that of community C was of a quiet, personal nature without so much attendant publicity. It was found that the umbrella privies built in communities A and C had gone to pieces in a year.

Dr. Stiles also presented a note on memory span studies in children. Of children from homes with privy and those from homes with sewer, it was found that the memory span of the last group compared with that of the first group as 14 to 10. For thirty-six boys and sixteen girls with light infestations with hookworm, the total memory span should have tested 343.24, and did in fact test 339, showing only a very slight variation below normal. For thirty-eight children infested with *Ascaris*, the total memory span should have been 245.23, and was in fact 250, a slight variation above the normal. For sixty-seven children infested with *Giardia* (*Lamblia*), the total memory span should have been 441.6, and was in fact 444, a slight variation above the normal. For fifty-five children infested with *Entameba coli*, the total memory span should have been 367.29, and was in fact 376. It therefore appears that while children from sanitary homes show a superiority over those from insanitary homes, so far as the memory span is concerned, of 14 to 10, the presence of slight infestations with hookworm, ascarids, *Giardia* or *Entameba coli* appear to bear no appreciable relation to the memory span.

MAURICE C. HALL, *Secretary*.

The twenty-ninth regular meeting of the society was held at the residence of Dr. Stiles Jan. 28, 1916, Dr. Stiles acting as host and Dr. Pfender as chairman.

Dr. Stiles presented a note in regard to cases of spurious parasitism. A slug, said to have been passed by a patient in Baltimore, and identified by Dr. Paul Bartsch as *Limax flava*, was shown to the society. In a second case, a physician had for years been regarded as presenting a case of multiple infestation with *Cysticercus cellulosae*, this being the diagnosis of the patient and of several other physicians. A physician who had examined the patient called in Dr. Stiles, and their examination disclosed the fact that the patient was addicted to the use of drugs administered by the usual hypodermic method. The patient's failure to use a properly sterilized needle had led to the formation of the small swellings which were present over the arms, legs and the portions of the body accessible to the needle, but significantly absent over the back. These swellings constituted the supposed cysticerci. One of these swellings when excised and sectioned showed connective tissue and pus. Dr. Stiles also noted the fact that the pulp vesicles of an orange had been sent to him with a diagnosis of *Dicrocoelium lanceatum*, and predicted that next spring and summer there would be the usual amount of hairs from the strawberry sent in as supposed specimens of pinworms and hookworms. He also recalled the sending in of a specimen, said to have been vomited by a boy and supposed to be parasitic, which proved to be a placental structure, apparently from a cat, and called attention in this connection to the historical *Spiroptera hominis*, which had proved to be the entrails, eggs and encapsulated nematode parasites of fish, which had evidently been introduced into the vagina by a hysterical woman patient.

Dr. Ransom presented the following notes on spurious parasitism: There was at one time in Washington a man who was accustomed to come to the Bureau of Animal Industry with an account of a peculiar affliction consisting in his being parasitized by insects which would suddenly appear in the skin,

quickly emerge and fly away. The man appeared sane on other topics. Dr. Ransom also noted a case in which supposed flukes were sent in as having been vomited by a boy. Examination showed them to be earthworms. In another case of a similar nature the supposed parasites proved to be two earthworms and a slug.

Mr. Crawley noted a case in which blood smears were sent in with the report that they showed blood parasites. These objects proved to be a common fungous structure which occurs in feces of all sorts almost anywhere.

Dr. N. A. Cobb gave a stereopticon demonstration, discussing about thirty species of nematodes found in the sand of slow filter beds from the filtration plants of various cities, and presenting three notes thereon:

Notes on Filter-Bed Nematodes.—Note 1.—Predaceous Nematodes

The discovery of nematodes in tap water led me to an investigation of conditions at filtration plants. Nematodes were found on the walls wet with spray at the flumes where the filtered water enters the city's supply. At the end of the period of use, usually a few weeks, the sand in the beds was found to contain hundreds of millions of nemas per acre in the top 3 inches. In one case, where the tale reached about one thousand million nemas per acre, nine tenths of the specimens were of one species, the predaceous *Mononchus longicaudatus* Cobb, which feeds on other nematodes, protozoa, etc., and hitherto known only from soil. This species is cosmopolitan. Another mononch, the *Mononchus papillatus* Bastian, I have shown, feeds on the citrus-root nema, an injurious parasite of various citrus trees, and there is a possibility that the filter-bed form may be economically serviceable in destroying injurious nemas. The filter-bed form is interesting from the fact that good preparations show that the esophagus is supplied with glandular structures opening into the lumen.

Two vegetarian species of *Monhystera* were found in the filter beds feeding on microbes and other organisms, and a species belonging to a new genus has the same food habits. *Ironus ignavus* Bastian and *Ironus longicaudatus* de Man, also found in large numbers in the filter beds, show in the cells of the intestinal walls doubly refractive granules which have also been found in the lumen of the intestine, indicative of a cannibalistic food habit. *I. ignavus* has an interesting egg, with peculiar chromatic elements scattered through its cytoplasm. In both forms the renette, hitherto undiscovered, is well developed and empties near the lips. Both have esophageal (salivary?) glands emptying into the pharynx.

Tripyla monhystera de Man is a very active, rapacious, carnivorous nema feeding on other nemas and on rotifers and protozoa, and is very common in filter beds. It suffers from what appears to be a protozoan disease, the protozoan usually invading it in the region of the tail, the invasion progressing most rapidly along the lateral fields. The affected nemas lose their normal activity and show signs of disease. The infection terminates, at least at times, in the death of the host.

NOTE 2.—Syngonism and Parthenogenesis; Cryptogenesis

Among these filter-bed nemas I have quite a complete series from bisexual species, through those showing obvious syngonism with prominent development of sperm in the gone followed by egg development, to those syngones in which the sperm development is rapidly accomplished and results in relatively inconspicuous though functional sperms. So complete is this series, ending in sperm discoverable with the utmost difficulty on account of minuteness, that

the fact that in any particular case the presence of sperm was not demonstrable, as, for instance, was the case in a species of *Ironus*, could not be regarded as proving its absence. Since in syngonism there is a single primordial gonocytic cell which by division gives rise to sperm and then to eggs in the same gone within a very short time, the idea is suggested that instead of this cell division producing these various elements and then a little later uniting them in the process of fertilization, the essential processes might occur in the earlier unicellular stage and the whole affair be consummated as a more nearly simultaneous instead of a consecutive process. This theory I suggest for consideration in connection with parthenogenesis. Such a method of reproduction, if it exists, I would denominate cryptogenesis.

NOTE 3.—*Revision of the Genus Cylandrolaimus*

Careful examination of a new species of *Cylandrolaimus* from the Washington filter beds has led to a more complete characterization of *Cylandrolaimus*, and a revision of the genus, as follows:

Cylandrolaimus de Man, 1884.—Small squat or meadow-land species, with naked, striated cuticle; cephalic setae, four, spreading, submedian; pharynx long, narrow, cylindrical, unarmed; lips rudimentary or none; labial papillae exceedingly minute; amphids circular, depressed; esophagus cylindroid, valveless, with well-developed cylindrical cardia; intestine thick walled, granular, not tessellated; tail moderately long, usually blunt, containing three caudal glands emptying through a plain, rounded, unarmed spinneret. Ovary single, outstretched; with a small branch on the other side of the vulva. Males rare or none, and, so far as known, having two equal, arcuate spicula, with very rudimentary accessory piece; male supplementary organ one, simple, slightly elevated, opposite the spicula; *C. communis* de Man denominated type species by de Man.

Key to Species Thus Far Referred to *Cylandrolaimus*

The last species (5, 6, 7 and 9) are not *cylandrolaimi*; the genus to which each may belong is suggested in parenthesis:

Bulb about pharynx, none; ovary one (except in No. 6); tail simply conoid; head rounded; amphids as wide as pharynx.....	<i>f-communis</i> de Man 1
Amphids half as wide as pharynx; ceph. setae half long as head is wide; oes. 20%; spin. symmetrical	- <i>f obtusus</i> n. sp. 2
Ceph. setae papilloid; oes, 14%; spinneret asymmetrical	? <i>melancholicus</i> de Man 3
Tail conoid, then cylindroid; head more or less truncate; pharynx twice long as head is wide; ceph. setae four or none; uterus and ovary simple; amphids minute or none; ovary reflexed; amph. small entering obliquely	(<i>Cylandrolaimus</i> ?)
..... (<i>Cylandrolaimus</i> ?)	' <i>f tristis</i> Ditlevsen 4
Ovary outstretched; no amphids, setae or spinneret	(Gen. nov.?)
..... (Gen. nov.?)	- <i>f macrurus</i> Daday 5
Uteri 2; ovaries reflexed; amphid a spiral	(<i>Plectus</i> ?)
..... (<i>Plectus</i> ?)	' <i>f aberrans</i> Micoletzky 6
Pharynx as long as head is wide; cephalic setae 6.....	(<i>Prismatolaimus</i> ?)
..... (<i>Prismatolaimus</i> ?)	- <i>f politus</i> Daday 7
Cephalic setae 4.....	- <i>f brachystoma</i> Hofmänner 8
Bulb of pharynx distinct, ovaries 2; setae none	(<i>Ethmolaimus</i> ?)
..... (<i>Ethmolaimus</i> ?)	? <i>lacustris</i> Hofmänner 9

C. obtusus n. sp. ♂ $\frac{3.2}{2.0}$ — $\frac{10}{2.6}$ — $\frac{22}{3.3}$ — $\frac{58.7}{3.5}$ — $\frac{87}{2.8}$ → .6 mm. Resembles *C. communis*, from which it differs in the form of the female sexual organs, the cephalic setae, and form and size of the amphids. Ventral excretory pore opposite the middle of the pharynx. Appears to be digonic, since the small outstretched posterior branch of the sexual organ appears to function as a testis. Habitat: sand-filter beds, Washington, D. C.

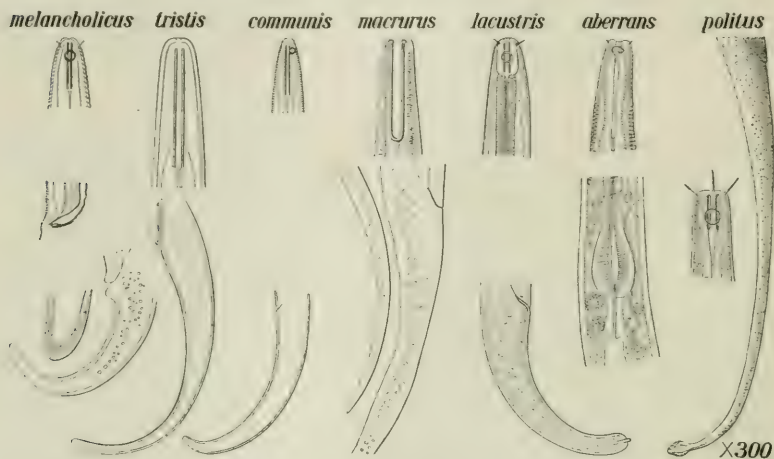


Fig. 1.—Heads and tails of species of *Cyndrolaimus* referred to in the key, reproduced from illustrations in the published descriptions of the species.

<i>melancholicus</i>	♂ $\frac{1.7}{1.7}$ — $\frac{?}{?}$ — $\frac{14}{3}$ — $\frac{53.71}{3.8}$ — $\frac{90}{2.5}$ → 1.3 mm.	<i>milnechlicus</i>	♂ $\frac{1.7}{1.7}$ — $\frac{?}{?}$ — $\frac{14}{3}$ — $\frac{M}{3.8}$ — $\frac{90}{2.5}$ → 1.1 mm.
<i>communis</i>	♂ $\frac{3.9}{1.8}$ — $\frac{11.7}{2.5}$ — $\frac{19.3}{3}$ — $\frac{56.21}{3.7}$ — $\frac{88.5}{2.3}$ → 64 mm.	<i>tristis</i>	♂ $\frac{3}{1.1}$ — $\frac{?}{?}$ — $\frac{26}{?}$ — $\frac{59.1219}{1.5}$ — $\frac{92.6}{1.3}$ → 1.8 mm.
<i>macrurus</i>	♂ $\frac{3.6}{2.1}$ — $\frac{?}{?}$ — $\frac{18.7}{3.8}$ — $\frac{51.8}{4.4}$ — $\frac{77}{2.5}$ → 1.4 mm.	<i>aberrans</i>	♂ $\frac{3.9}{1.9}$ — $\frac{9}{2.4}$ — $\frac{15}{2.8}$ — $\frac{44.21}{3}$ — $\frac{81}{1.7}$ → 1.1 mm.
<i>politus</i>	♂ $\frac{1.4}{1.5}$ — $\frac{6.1}{2.5}$ — $\frac{16}{3.4}$ — $\frac{60}{3.4}$ — $\frac{81}{1.8}$ → 1.1 mm.	<i>lacustris</i>	♂ $\frac{2.8}{2.6}$ — $\frac{?}{?}$ — $\frac{16.6}{?}$ — $\frac{50.4}{2.9-5}$ — $\frac{84.5}{3.2}$ → .7 mm.

Fig. 2.—The formulae of the species referred to in the key.

Dr. Pfender presented a note in regard to a patient who thought that he had a tapeworm. Radiographs presented by Dr. Pfender showed that the symptoms which the patient referred to were due to nephrolithiasis. A nephrectomy was performed and the stones, one large one and numerous smaller ones, which had been found in the kidneys, were exhibited.

MAURICE C. HALL, Secretary.

REVIEWS AND NOTES

DIE TIERISCHEN PARASITEN DES MENSCHEN

1. TEIL: NATURGESCHICHTE DER TIERISCHEN PARASITEN DES MENSCHEN VON DR. MAX BRAUN. Fünfte, vermehrte und verbesserte Auflage. 560 pp. 407 text figures. Curt Kabitzsch Verlag in Würzburg. 1915. 13 mk.; geb. 15.50 mk.

The appearance of a new edition of the well-known and highly prized text by Braun is deserving of more than ordinary notice here. It is seven years since the fourth edition was published and the literature on parasitology which, especially among Protozoa, has modified and extended the world's knowledge of this important field, may fairly be said to have doubled in that short interval of time. The last (fourth) edition of Braun's work saw the addition of a clinical-therapeutic section which in this edition has been expanded to a second part, the separate appearance of which is promised at an early date. Unquestionably it was the rapid growth in materials demanding consideration which has led to the separation of the newly introduced section as an independent item; for the first part, which covers only the structure, life history, distribution and classification of the species parasitic in man, now utilizes 560 pages—or more than twice the compass of the entire work in the third edition.

The increase in size is also accompanied by marked changes in form such as to allow of more extended discussion in the same space. The type page is both larger and wider. More of the present volume has been thrown into fine type and other means of condensation have been employed freely in the effort to bring present knowledge into a reasonable compass. Unfortunately in this process the author was compelled to reduce the introduction considerably in extent, a change which every one must view with real regret for Professor Braun is an artist in presenting concisely and clearly any discussion of general principles and many generations of students have read with profit and delight his opening chapter on Parasitism in General. The general discussions with which the account of each group was introduced have also suffered somewhat in the process of condensation.

Even with all this trimming the text has grown fully twenty per cent. in volume. New material is in evidence everywhere. The new edition is in fact a real revision and not a mere reprinting with minor textual modifications. The author has added a very considerable number of new species which have been discovered since the appearance of the last edition or which since then have been found to be of significance to man. This increase runs from ten to thirty per cent. in different groups.

The plan adopted in the previous edition of grouping the important references to the literature in a section at the close of the text proper has been followed here more consistently. This list, though sharply scanned, has increased greatly in extent and now covers 110 text pages. It is notably fairer than most foreign lists in its treatment of American work and is thoroughly up to date. A few typographical errors were noted and some curious abbreviations in titles of English articles. Unfortunately the references on topics in the last 25 pages are printed in the text after the manner of earlier editions and not brought together in the bibliographic list. This detracts somewhat from the character of the work and the record is also not so good. Yet, one may say confidently that it is the best reference list available in this field.

The illustrations are frequent, good, and about one fourth of them new. Some ancient favorites that are not very accurate still occupy their historic

places. Thus the figures of *Demodex* and of the female *Ixodes ricinus* are little worthy of a place in such a work. But on the whole the work is better and more profusely illustrated than our own texts in biological science.

The section on Protozoa has perhaps been modified most of all. The system is greatly expanded and one notes that the Cnidosporidia have been exalted to the rank of a class, a conspicuous departure from the time honored division of this phylum into four classes. By the removal of the discussions concerning insect vectors (mosquitoes, biting flies, etc.) to the chapter on insects the apparent increase in size is not marked superficially, but the space gained in this way is more than filled by data on the group proper. New species, new data on morphology, life history, and biology, as well as recent experimental work, and new figures are prominent in this section.

In the chapter on Trematodes the author has introduced a systematic outline prepared by Odhner and embodying the recent important researches of that distinguished investigator on the relationships of the various groups of flukes. This system marks a distinct advance in the direction of a natural classification based on comparative anatomy and follows the line of attack formulated in Looss' epochal studies on the natural classification of the Trematodes. It is interesting to note that even in this long known and much studied group, the text lists seven species out of twenty-one that were not mentioned in the previous edition, and that the accounts of species formerly listed almost all have been radically revised in correspondence with recent discoveries concerning them. The author displays commendable conservatism in refusing to follow extreme modifications in nomenclature and yet he has not hesitated here or elsewhere in the volume to use new names when their establishment rests upon adequate study and morphological demonstration.

Among the Cestodes which furnish the fewest species to the list of human entozoa there are less changes to record. The older species have undergone little alteration though one name, *Hymenolepis lanceolata*, has been eliminated on the basis of error as indicated by Fuhrmann in 1908 in a note generally overlooked. But it will surprise even those somewhat familiar with the literature to find that among the twenty-seven species of tapeworms listed in this work seven are new within the last seven years.

No group of helminthes has undergone greater changes in recent years and is still in greater need of revision on the basis of such studies as Looss, Lühe, and Odhner have made among Trematodes, than the Nematodes. Thanks especially to Goldschmidt, our knowledge of nematode structure has been greatly advanced and full use is made of this advance in the work under review. The system is still a disconnected series of "families," based on factors very dissimilar in character and value, representing thus sometimes a small group of closely related species and in other cases a large mass of anatomically variant forms drawn together by artificial definitions. Some slight progress has been made in the solution of the difficulties by the pioneer work of Railliet and Henry which has been used by Braun. Nevertheless the "system" remains little more than a list. Under individual species Braun has added much new and important material, such as the work of Fülleborn on filarial life history, of Looss on the hookworms and other species, and of many others. A good many new species and also some new names greet the reader in this section. Fortunately figures and descriptions are now adequate in the main for an understanding of the species and for their differentiation; this which has not been true in earlier works, will do much to clear up the confusion which exists in this group.

Among the Arthropoda (mostly ectoparasitic mites and insects) one notes a fuller treatment which conforms to the now fully demonstrated rôle of these forms in the transmission of protozoal diseases. The increase is due partly, as already noted, to the transfer of such materials as in earlier editions were found

under other headings. This change has made distinctly for the unity and clarity of the work and was necessitated further by the demonstrated agency of the single form in the transmission of various parasitic organisms, e. g., the mosquito as the inoculator of several protozoa and filariae. But beyond this the arthropod section contains much new material. Especial mention should be made of the fine new figures and the carefully collated data for differential diagnosis of important species. Of course the accumulated knowledge in this part of the field is great, as exemplified by recent works devoted exclusively to it, and Braun has not attempted to include all. But as a summary this part must be recognized as a real success and a great advance over the distinctly inadequate treatment accorded this phase of the subject in earlier editions.

All in all the new edition represents a most valuable contribution to helminthological literature. It is a worthy production of the famous head of the Königsberg school of parasitologists and justly entitles him again to the congratulations and thanks of other workers in this field.

Doctor Jesus Rafael Risquez of Caracas has published an interesting study of nineteen cases of the blood fluke (*Schistosoma mansoni*) observed in eighty-six autopsies in Venezuela, fourteen of which came from the white race, none from the indian or negro, and five from half breeds; in large part the patients were born in Caracas.

The Report of the United Fruit Company's Medical Department for 1914 was reviewed in this Journal last December. The Report for 1915 confirms in essential details the conditions regarding the occurrence of human parasites on the shores of the Caribbean which were taken from the previous report and embodied in the tables of the review cited.

HIBERNATION OF MUSCA DOMESTICA

In 1913 Dr. Henry Skinner challenged the commonly accepted belief that adult house flies remained dormant throughout the Winter months. He even went so far as to say tentatively that house flies passed the Winter in the pupal stage and in no other way. Dr. Johannsen's observations at Ithaca tended to confirm Dr. Skinner's conclusion insofar as it applied to conditions in the latitude of New York State.

In January of this year an instructor in the Department, Mr. W. L. Chandler, observed several adult specimens of *Musca domestica* in the sub-basement of Roberts Hall, one of our University buildings. I have observed others in the sub-basement and around in the buildings even at this late date (April 7). These were remote from breeding places and there seems no possibility that they hibernated in the pupal stage.

WILLIAM A. RILEY

In a recent important paper Crawley has shown that when mice are fed material containing the so-called spores of *Sarcocystis muris* invasion of intestinal epithelial cells by the parasites takes place within two hours. This phenomenon is most favorably studied in the last inch or two of the small intestine. Within the cells, the parasites rapidly separate into two categories, the latter history of which shows them to be males and females.

In the male, development takes the form of a notable increase in the size of the nucleus, correlated with a loss of most if not all of the cytoplasm. Various internal changes take place within this enlarged nucleus, and eventually the chromatin becomes divided into clusters of minute granules, grouped around the periphery. These granular clusters solidify into compact balls, which elongate and produce the microgametes.

In the females, the changes are not so conspicuous. The cell becomes shorter and broader than the original spore, but there is no loss of cytoplasm nor any conspicuous enlargement of the nucleus. The nuclear chromatin remains concentrated in a large karyosome.

This sexual evolution is completed in from 9 to 18 hours, after which fertilization takes place. The further history of the zygote has not been followed.

INDEX TO VOLUME II

	PAGE
Acanthocephala from Fresh-Water Hosts, Seasonal Distribution of Some..	106
Arachnoid, <i>Pneumonyssus foxi</i> nov. sp., Parasitic in the Lung of a Monkey (<i>Macacus rhesus</i>)	37
Archives de Parasitologie (note).....	148
Archivos Brasileiros de Medecina (review).....	148
Are Sarcosporidia Aberrant Forms of Cnidosporidia of Invertebrates?....	126
Arhythmorhynchus, Revision of the Genus, with Descriptions of Two New Species from North American Birds.....	167
Arthropoda, Some New Gregarine Parasites from.....	27
Barrett, M. T., see Smith, Allen J., and M. T. Barrett.	
Bartonella, Note on the Etiology of Verruga as Deduced from a Study of the Asexual Stages of.....	143
Book Reviews, see Reviews.	
Braun, Max: Naturgeschichte der tierischen Parasiten des Menschen, 1. Teil (review)	201
Brazil, Medical Zoology in (book reviews).....	148
<i>Catostomus commersonii</i> , On the Occurrence of a Trypanoplasm, Probably <i>Trypanoplasma borreli</i> Laveran et Mesnil, in the Blood of the Com- mon Sucker	1
Cattle Tick, <i>Margaropus annulatus</i> , Note on the Stage of <i>Piroplasma</i> <i>bigeminum</i> which Occurs in the.....	87
Cestode Cysts from Muskrat.....	46
Cestodes, Polyradiate, Two New Cases of, with a Summary of the Cases Already Known	7
Chidester, F. E.: Sarcophagid Larvae from the Painted Turtle.....	48
Cnidosporidia of Invertebrates, Are Sarcosporidia Aberrant Forms of....	126
Cort, William Walter: Egg Variation in a Trematode Species.....	25
Crawley, Howard: Note on the Stage of <i>Piroplasma bigeminum</i> which Occurs in the Cattle Tick, <i>Margaropus annulatus</i>	87
Crustacea, Marine, Three New Gregarines from.....	129
<i>Cryptobranchus allegheniensis</i> , <i>Filaria cingula</i> Parasitic in the Skin of....	74
<i>Cylindrotaenia americana</i> nov. spec. from the Cricket Frog.....	181
Cysts, Cestode, from Muskrat.....	46
Effect of Tick Bites on Man.....	193
Egg Variation in a Trematode Species.....	25
Encysted Larva of the Lung Distome, Some Notes on.....	175
<i>Endamoeba gingivalis</i> (Gros) and <i>Endamoeba histolytica</i> Schaudinn, Fur- ther Note upon Comparison of.....	54
Etiology of Verruga as Deduced from a Study of the Asexual Stages of Bartonella, Note on the.....	143
Famille des Thelaziidae.....	99
Fantham, H. B., and Annie Porter: Significance of Certain Natural Flagel- lates of Insects in the Evolution of Disease in Vertebrates.....	149
<i>Filaria cingula</i> Parasitic in the Skin of <i>Cryptobranchus allegheniensis</i>	74
Flagellates of Insects, Significance of Certain, in the Evolution of Disease in Vertebrates	149
Foster, Winthrop D.: Two New Cases of Polyradiate Cestodes, with a Summary of the Cases Already Known.....	7
Further Note upon Comparison of <i>Endamoeba gingivalis</i> (Gros) and <i>Endamoeba histolytica</i> Schaudinn.....	54
Galli-Valerio, B.: Are Sarcosporidia Aberrant Forms of Cnidosporidia of Invertebrates?	126
Gongylonema in the Rôle of a Human Parasite.....	119
<i>Gongylonema scutatum</i> , Life History of.....	80
Gregarine Parasites from Arthropoda, Some New.....	27
Gregarines, Three New, from Marine Crustacea.....	129
Hall, Maurice C., see Ransom, Brayton H., and Maurice C. Hall.	

Harvard School of Tropical Medicine. Report of First Expedition to South America (review).....	147
Helminthological Society of Washington, Proceedings.....	93, 195
Hermis, William B.: Pajaroello Tick (<i>Ornithodoros coriaceus</i> Koch) with Special Reference to Life History and Biting Habits.....	137
Infusorian Parasite in Sand Fleas, New.....	145
Insect Vector of Uta, a Peruvian Disease.....	67
Intermediate Hosts of the Lung Distome, <i>P. westermanii</i> Kerbert.....	111
Jewell, Minna E.: <i>Cylindrotaenia americana</i> nov. spec. from the Cricket Frog.....	181
<i>Katayama nosophora</i> as a Host (note).....	50
Krecker, Frederic H.: <i>Filaria cingula</i> Parasitic in the Skin of <i>Cryptobranchus allegheniensis</i>	74
Larvae, Sarcophagid, from the Painted Turtle.....	48
Life History of <i>Gongylonema scutatum</i>	80
Linton, Edwin: Cestode Cysts from Muskrat.....	46
<i>Macacus rhesus</i> , <i>Pneumonyssus foxi</i> nov. sp., Arachnoid Parasitic in the Lung of a Monkey.....	37
Man, Parasites of:	
Effect of Tick Bites on Man.....	193
Encysted Larva of the Lung Distome.....	175
Further Note upon Comparison of <i>Endamoeba gingivalis</i> (Gros) and <i>Endamoeba histolytica</i> Schaudinn.....	54
Gongylonema in the Rôle of a Human Parasite.....	119
Insect Vector of Uta, a Peruvian Disease.....	67
Intermediate Hosts of the Lung Distome, <i>P. westermanii</i> Kerbert....	111
Naturgeschichte der tierischen Parasiten des Menschen, 1. Teil (review)	201
Pajaroello Tick (<i>Ornithodoros coriaceus</i> Koch) with Special Reference to Life History and Biting Habits.....	137
Rate of Growth of the Beef Tapeworm in Human Beings (note)....	98
<i>Schistosoma japonicum</i> (note).....	50
<i>Schistosoma mansonii</i> (note).....	203
<i>Margaropus annulatus</i> , Note on the Stage of <i>Piroplasma bigeminum</i> which Occurs in the Cattle Tick.....	87
Mavor, J. W.: On the Occurrence of a Trypanoplasma, Probably <i>Trypanoplasma borreli</i> Laveran et Mesnil, in the Blood of the Common Sucker, <i>Catostomus commersonii</i>	1
McCaffrey, D.: Effect of Tick Bites on Man.....	193
Memorias do Instituto Oswaldo Cruz (review).....	148
<i>Musca domestica</i> , Hibernation of (note).....	203
Muskrat, Cestode Cysts from.....	46
New Infusorian Parasite in Sand Fleas.....	145
Notes.....	50, 96, 147, 201
Occurrence of a Trypanoplasma, Probably <i>Trypanoplasma borreli</i> Laveran et Mesnil, in the Blood of the Common Sucker, <i>Catostomus commersonii</i>	1
<i>Ornithodoros coriaceus</i> Koch, Pajaroello Tick, with Special Reference to Life History and Biting Habits.....	137
Pajaroello Tick (<i>Ornithodoros coriaceus</i> Koch) with Special Reference to Life History and Biting Habits.....	137
<i>Paragonimus westermanii</i> Kerbert, On the Intermediate Hosts of the Lung Distome.....	111
<i>Piroplasma bigeminum</i> which Occurs in the Cattle Tick, <i>Margaropus annulatus</i> , Note on the stage of.....	87
<i>Pneumonyssus foxi</i> nov. sp., Arachnoid Parasitic in the Lung of a Monkey (<i>Macacus rhesus</i>).....	37
Polyradiate Cestodes, Two New Cases of, with a Summary of the Cases Already Known.....	7
Porter, Annie, see Fantham, H. B., and Annie Porter.	
Prowazek, Professor von, Sketch of.....	51
<i>Psoroptes cuniculi</i> (note).....	98
Railliet, A.: Famille des Thelaziidae.....	99
Ransom, Brayton H., and Maurice C. Hall: Life History of <i>Gongylonema scutatum</i>	80

	207
	PAGE
Report of First Expedition to South America (review).....	147
Reviews:	
Archivos Brasileiros de Medecina.....	148
Memorias do Instituto Oswaldo Cruz.....	148
Naturgeschichte der tierischen Parasiten des Menschen, 1. Teil.....	201
Report of First Expedition to South America. Harvard School of Tropical Medicine	147
Revision of the Genus <i>Arhythmorhynchus</i> , with Descriptions of Two New Species from North American Birds.....	167
<i>Sarcocystis muris</i> (note).....	203
<i>Sarcocystis tenella</i> Railliet, Some Notes and Experiments on.....	20
Sarcophagid Larvae from the Painted Turtle.....	48
<i>Schistosoma japonicum</i> (note).....	50
<i>Schistosoma mansoni</i> (note).....	203
Scott, John W.: Some Notes and Experiments on <i>Sarcocystis tenella</i> Railliet	20
Seasonal Distribution of Some Acanthocephala from Fresh-Water Hosts..	106
Significance of Certain Natural Flagellates of Insects in the Evolution of Disease in Vertebrates.....	149
Smith, Allen J., and M. T. Barrett: Further Note upon Comparison of <i>Endamoeba gingivalis</i> (Gros) and <i>Endamoeba histolytica</i> Schaudinn...	54
Some New Gregarine Parasites from Arthropoda.....	27
Some Notes and Experiments on <i>Sarcocystis tenella</i> Railliet.....	20
Some Notes on the Encysted Larva of the Lung Distome.....	175
Strong, Richard P.: Report of First Expedition to South America. Har- vard School of Tropical Medicine (review).....	147
Stunkard, Horace W.: Notes on the Trematode Genus <i>Telorchis</i> with Descriptions of New Species.....	57
<i>Taenia saginata</i> , rate of growth (note).....	98
Tapeworm, rate of growth (note).....	98
<i>Telorchis</i> , Notes on the Trematode Genus, with Descriptions of New Species	57
Thelaziidae, Famille des.....	99
Three New Gregarines from Marine Crustacea.....	129
Tick Bites on Man, Effect of.....	193
Townsend, Charles H. T.: Insect Vector of Uta, a Peruvian Disease.....	67
Note on the Etiology of Verruga as Deduced from a Study of the Asexual Stages of Bartonella.....	143
Trematode Genus <i>Telorchis</i> , Notes on the, with Descriptions of New Species	57
Trematode Species, Egg Variation in.....	25
<i>Trypanoplasma borreli</i> Laveran et Mesnil, in the Blood of the Common Sucker, <i>Catostomus commersonii</i> , On the Occurrence of.....	1
Two New Cases of Polyradiate Cestodes, with a Summary of the Cases Already Known	7
United Fruit Company, Medical Report (note).....	96, 203
Uta, Insect Vector of, a Peruvian Disease.....	67
Van Cleave, H. J.: Revision of the Genus <i>Arhythmorhynchus</i> , with Descriptions of Two New Species from North American Birds.....	167
Seasonal Distribution of Some Acanthocephala from Fresh-Water Hosts	106
Verruga, Note on the Etiology of, as Deduced from a Study of the Asexual Stages of Bartonella.....	143
Ward, Henry B.: Gongylonema in the Rôle of a Human Parasite.....	119
Watson, Minnie E.: New Infusorian Parasite in Sand Fleas.....	145
Some New Gregarine Parasites from Arthropoda.....	27
Three New Gregarines from Marine Crustacea.....	129
Weidman, Fred D.: <i>Pneumonyssus foxi</i> nov. sp., Arachnoid Parasitic in the Lung of a Monkey (<i>Macacus rhesus</i>).....	37
Yoshida, Sadao: Intermediate Hosts of the Lung Distome, <i>P. westermanii</i> Kerbert	111
Some Notes on the Encysted Larva of the Lung Distome.....	175

ERRATA

For the table on page 199, Vol. II, No. 4, substitute the following:

Bulb about pharynx, none; ovary one (except in No. 6)	
Tail simply conoid; head rounded	
Amphids as wide as pharynx.....	f- <i>communis</i> de Man 1
Amphids half as wide as pharynx	
Ceph, setae half long as head is wide; oes. 20%; spin. symmetrical	
-f <i>obtus</i> n. sp.	2
Ceph. setae papilloid; oes. 14%; spinneret asymmetrical	
? <i>melancholicus</i> de Man	3
Tail conoid, then cylindroid; head more or less truncate	
Pharynx twice long as head is wide; ceph. setae four or none	
Uterus and ovary simple; amphids minute or none	
Ovary reflexed; amph. small entering obliquely (<i>Cylindrolaimus</i> ?).....	
'f' <i>tristis</i> Ditlevsen	4
Ovary outstretched; no amphids, setae, or spinneret (gen. nov. ?)	
-f <i>macrurus</i> Daday	5
Uteri 2; ovaries reflexed; amphid a spiral (<i>Plectus</i> ?).....	
'f' <i>aberrans</i> Micoletzky	6
Pharynx as long as head is wide	
Cephalic setae 6.....(<i>Prismatolaimus</i> ?)-f <i>politus</i> Daday	7
Cephalic setae 4.....-f <i>brachystoma</i> Hofmänner	8
Bulb of pharynx distinct; ovaries 2; setae none (<i>Ethmolaimus</i> ?)	
? <i>lacustris</i> Hofmänner	9

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CONTENTS OF VOLUME III

SEPTEMBER, 1916. NUMBER 1

	PAGE
CONTRIBUTIONS TO THE STUDY OF PARASITIC PROTOZOA. III. NOTES ON MYXOSPORIDIA FOUND IN SOME FRESH-WATER FISHES OF JAPAN, WITH THE DESCRIPTION OF THREE NEW SPECIES. ROKUSABURO KUDO.....	3
(With four text figures)	
NOTES ON TWO FREE-LIVING LARVAL TREMATODES FROM NORTH AMERICA. HENRY B. WARD.....	10
(With one plate)	
ON THE ANATOMY AND RELATIONSHIPS OF SOME NORTH AMERICAN TREMATODES. HORACE W. STUNKARD.....	21
DAUERCYSTFORMATION OF <i>Trichomonas intestinalis</i> . KENNETH M. LYNCH..	28
(With two text figures)	
NOTES ON TWO CESTODES FROM THE SPOTTED STING-RAY. EDWIN LINTON..	34
(With one plate and two text figures)	
A CASE OF THE OCCURRENCE OF <i>Ascaris triquetra</i> SCHRANK IN DOGS. A. C. WALTON.....	39
(With six text figures)	
REVIEWS AND NOTES.....	42

DECEMBER, 1916. NUMBER 2

THE EFFECTS OF RADIATION ON THE DEVELOPMENT OF <i>Trichinella spiralis</i> , WITH RESPECT TO ITS APPLICATION TO THE TREATMENT OF OTHER PARASITIC DISEASES. E. E. TYZZER AND JAMES A. HONEIJ.....	43
(With one plate)	
NOTES ON SOME NEMATODES FROM FRESH-WATER FISHES. HENRY B. WARD AND THOMAS B. MAGATH.....	57
(With one plate)	
OBSERVATIONS ON POLYCYSTID GREGARINES FROM ARTHROPODA. MINNIE E. WATSON	65
(With one plate)	
ON A TREMATODE LARVA ENCYSTED IN A CRAB, <i>Helice tridens</i> (DE HAAN). SADA0 YOSHIDA.....	76
(With two text figures)	
<i>Cytolichus peurosci</i> , A NEW ARACHNOID PARASITE FOUND IN THE DISEASED LUNGS OF A PRAIRIE DOG, <i>Cynomys ludovicianus</i> . FRED D. WEIDMAN	82
(With two plates)	
BOOK REVIEW AND NOTE.....	90

MARCH, 1917. NUMBER 3

PAGE

ON THE SPOROZOON PARASITES OF THE FISHES OF WOODS HOLE AND VICINITY. I. FURTHER OBSERVATIONS ON <i>Myxobolus musculi</i> FROM FUNDULUS. C. W. HAHN.....	91
(With three text figures)	
NOTES ON THE CERCARIAE OF THE BITTER ROOT VALLEY, MONTANA. ERNEST CARROLL FAUST.....	105
(With one plate)	
THE DEVELOPMENT OF GREGARINES AND THEIR RELATION TO THE HOST TIS- SUE: (I) IN <i>Stenophora lactaria</i> WATSON. MINNIE WATSON KAMM	124
(With two plates)	
THE CERCARIAE OF NATAL. F. G. CAWSTON.....	131
NOTE ON A SPECIES OF NOSEMA INFECTING <i>Attacus cynthia</i> DRURY. SHIGETANE ISHIWATA.....	136
(With eight text figures)	
NOTES ON <i>Porocephalus globicephalus</i> . THESLE T. JOB AND A. R. COOPER..	138
BOOK REVIEWS.....	139
NOTES	141

JUNE, 1917. NUMBER 4

<i>Endamoeba buccalis</i> . I. ITS MULTIPLICATION AND PERIODICITY. NADINE NOWLIN	143
(With one text figure)	
ON THE SPOROZOON PARASITES OF THE FISHES OF WOODS HOLE AND VICINITY. II. ADDITIONAL OBSERVATIONS ON <i>Myxobolus musculi</i> OF FUNDULUS AND A NEARLY RELATED SPECIES, <i>M. pleuronectidae</i> OF <i>Pseudopleuronectes</i> <i>americanus</i> . C. W. HAHN.....	150
(With one plate)	
CONTRIBUTIONS TO THE STUDY OF PARASITIC PROTOZOA. II. <i>Myxobolus</i> <i>toyamai</i> NOV. SPEC., A NEW MYXOSPORIDIAN PARASITE IN <i>Cyprinus</i> <i>carpio</i> L. ROKUSABURO KUDO.....	163
(With two plates)	
THE OCCURRENCE OF <i>Bothriocephalus liguloides</i> LEUCKART, WITH ESPECIAL REFERENCE TO ITS DEVELOPMENT. SADA O YOSHIDA.....	171
(With one text figure)	
A FURTHER NOTE ON THE LIFE-HISTORY OF <i>Gongylonema scutatum</i> . BRAYTON H. RANSOM AND MAURICE C. HALL.....	177
NOTES	182

The numbers of Volume III of the JOURNAL OF PARASITOLOGY were mailed as follows:

No. 1. Nov. 22, 1916.
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Volume III

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Volume 3

SEPTEMBER, 1916

Number 1

CONTRIBUTIONS TO THE STUDY OF PARASITIC PROTOZOA. III.

NOTES ON MYXOSPORIDIA FOUND IN SOME FRESH-WATER FISHES OF
JAPAN, WITH THE DESCRIPTION OF THREE NEW SPECIES
(WITH FOUR TEXT FIGURES)

ROKUSABURO KUDO

THE IMPERIAL SERICULTURAL EXPERIMENT STATION, NAKANO, TOKYO

In a former paper (1915) I described from a morphological as well as developmental point of view, a new species (*Myxobolus toyamai* Kudo) from the branchial lamellae of a carp. The present paper is the result of study upon Myxosporidia found since that time. I am now working on the life-histories of the new species described below with the hope of reporting them later.

1. *Myxosoma dujardini* Thel.

Eight cysts (the largest being 200μ in diameter) of round shape, were found in the branchial lamellae of a carp 23 cm. in length. The seat of the parasite was, as in *Myxobolus toyamai*, the connective tissue of the gill-filament.

The results of observations upon the spore coincide for the most part with the description of Thélohan (1895). I wish, however, to give here details about the polar capsule and the polar filament, as Thélohan failed to mention them: length and breadth of the polar capsule $6-7\mu$ and about 2μ , respectively, and the length of the polar filament about 70μ .

2. *Zschokkella acheilognathi* n. sp.

Vegetative form. Large ones are generally visible to the naked eye as small, opaque, more or less regular, usually subspherical masses, occupying various parts of the gallbladder and especially of the gall-duct (Fig. 1). The size varies with age up to a maximum length of 720μ by a breadth of 550μ , and the thickness of one individual is about uniform throughout, but in many specimens it differs from 5 to 30μ according to the size of the myxosporidium. Their bodies are very flexible and easily doubled up, representing, in sections, various forms. The vegetative stage in sections resembles much that of *Sphaeromyxa*

hellandi, observed by Auerbach (1912), both in form and structure. The body is colorless in both young and old. In its fresh condition the protoplasm can be seen to be clearly differentiated into finely granulated reticular ectoplasm and greatly vacuolated endoplasm. In the younger form (15 to 30μ in greatest diameter) lobose pseudopodia are well developed. The myxosporidium moves about more or less actively in the bile by the constant emission of pseudopodia. No clear evidence of the active emission of pseudopodia exists in older individuals. The ectoplasm of some more advanced specimens shows in section two structures; the outer layer, comparatively thin but uniformly about 2μ in thickness, presents very fine striations, while the remaining part is finely alveolated, having an average thickness of 6 to 8μ .

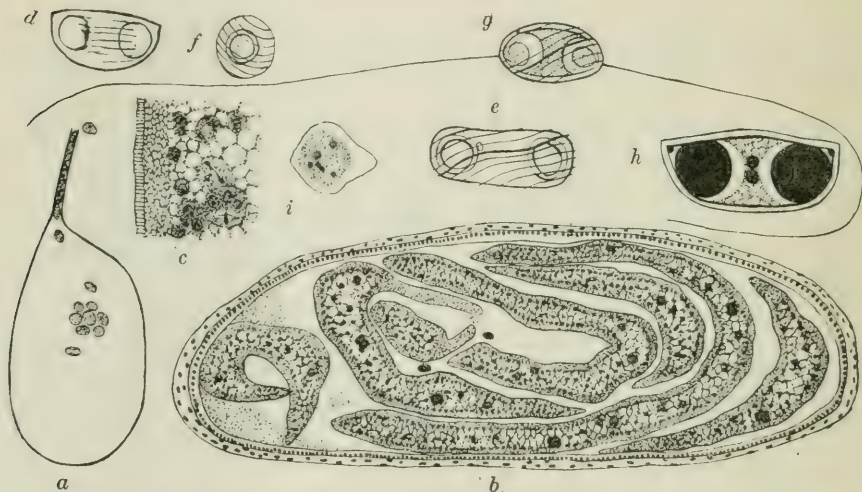


Fig. 1.—*Zschokkella acheilognathi* n. sp. *a*, Gall-bladder of *Acheilognathus* with many myxosporidia, $\times 10$; *b*, oblique cross section of the infected gallduct, $\times 200$; *c*, part of the cross section of the parasite, showing the differentiation of the protoplasm, $\times 1,000$; *d*, to *g*, spores: in *g*, filaments are extruded, $\times 1,500$; *h*, stained spore, about $\times 2,250$; *k*, young myxosporidium, $\times 1,000$.

Auerbach (1910b) seems to have observed similar structure in the outer layer of the ectoplasm in *Sphaeromyxa hellandi*, sketching a surface view of an individual fixed in formol. It takes stains much more deeply than the endoplasm, so that in sections it shows clear differentiation of the protoplasm much better than was shown in *Myxobolus toyamai*. The endoplasm contains vegetative nuclei as well as generative nuclei in several stages of spore formation. It is polysporous, according to the observation made up to the present time. In this regard, it is quite different from *Zschokkella hildae*, in which, after Auerbach (1910a), single and double spore formation occurs.

Spore. Generally oval with round poles, very often more or less

hemispherical, somewhat attenuated symmetrically at both ends of the flat side of the spore. Several modifications in form and size, however, are also found in the present case as in other forms. It is usually 10 to 14μ long by 6 to 7μ wide. The thickness is almost equal to the width. Shell bivalve; the line of junction being oblique to the longitudinal axis of the spore. Parallel to the line of junction, fine striations run longitudinally on the spore coat. A polar capsule, round in shape, with a diameter of 2 to 3μ , at each end of the spore, takes stains very deeply. Polar filaments were easily extruded by the application either of mechanical pressure or KOH-solution, and are well stained after my method (1913). The fully extruded polar filaments were 65 to 70μ long.

Habitat. This species is found quite abundantly in the gallbladder of *Acheilognathus lanceolatum* Temm. et Schl., commonly found in brooks in the vicinity of Tokio. Out of twenty-four fish (8 to 12 cm. long) examined in May, 1915, twenty-one were found to have harbored the parasite; thus the rate of the infection rises above 80 per cent. Matured, large vegetative forms were very often found in great numbers in the gallduct, while in the gallbladder of the same host I could find only a small number of isolated spores. Klokacewa (1914) described a somewhat similar form of spore from *Carassius vulgaris*, without finding the vegetative form. The species in question, though its vegetative form differs apparently from that of *Zschokkella hildae* seems to belong to that genus. Up to the present time two species of the genus have been reported, that is, *Zsch. hildae* and *Zsch. nova*, since Auerbach (1910) created it. The former apparently differs from the present form in several points. Now the dimensions of the spore of the latter seem to correspond very nearly to the myxosporidium in question. As it lacks, however, all other details, it is impossible to make accurate identification, so I treat this species as a new one, calling it *Zschokkella acheilognathi*.

3. *Myxobolus fuhrmanni* Auer

Isolated spores of this form occur very often in the bile of the loach (*Misgurnus anguillicaudatus*). The vegetative form has not yet been found by me. The description of the spore by Auerbach (1909) coincides well with my observations, except that I found the thickness of the spore coat to be uniform, whereas Auerbach's observation was in effect that the shell is especially thick at the posterior end of the spore. I wish to mention that the length of the polar filament in the present case is 100μ . Nearly 50 per cent. of the said fish, examined in September, 1915, were infected by this *Myxobolus*. In all cases, however, the infection seems to be carried to a very slight degree.

4. *Myxidium* sp.

This form, together with the following two new species, are also found in the gallbladder of the loach. The vegetative form has not yet been observed. Two per cent. of the fish studied in September, 1915, were infected. The spores are mostly found separated from each other, floating in the bile. One side of the spore is, in most cases, more convex than the other. The sporoplasm usually occupies the whole inner space of the spore, except the polar capsules, and shows fine granulations in the natural condition as in the case of *Myxidium giardi* or *Myxobolus pfeifferi*, and also shows a fine alveolar structure in stained preparations. It contains two nuclei of almost equal size. Shell bivalve, the line of junction of which is straight. On the surface of the shell, fine striations run longitudinally parallel to the line of junction. The dimensions are: length, 15 to 18 μ , breadth 6 to 7 μ , length of polar capsule 7 to 8 μ , and that of polar filament 60 to 70 μ .



Fig. 2.—*Myxidium* sp. *a*, stained spore, $\times 1,750$; *b*, spore with extruded polar filaments, $\times 1,000$.

Ishii (1915) recently described a new species, *Myxidium anguillae*, from an eel. The form in question apparently differs from any of the species reported up to the present time. I hope to identify the species after studying it more closely.

5. *Chloromyxum misgurni* n. sp.

Vegetative form. Mostly round, often of irregular form. From a side view it assumes a semicircular shape. From the more or less flat surface many fine root-like pseudopodia extend. They are more clearly visible in younger specimens, where the spore formation has not yet begun. There is no clear differentiation of protoplasm. It is finely vacuolated on the whole. With the pseudopodia, the myxosporidium probably creeps along the surface of the epithelial layer of the gallbladder, so that many individuals in different stages of development are found in section preparations, closely attached to the epithelial cells with their peculiar pseudopodia. The size varies with age, the largest being 50 μ in greatest diameter, with the maximum thickness of 20 μ . Individuals with six to eight spores are of common occurrence; those with twelve to sixteen spores, however, occur rarely, and it is very seldom that only two spores are found in one myxosporidium.

Spore. Spherical, slightly attenuated at the anterior end. Shell

bivalve; the line of junction straight. Parallel to the ridge which marks the line of junction very clearly, run fine longitudinal striations. Four polar capsules are situated in the anterior end. In the finely granulated sporoplasm two nuclei of equal size are found. The dimensions of the spore are: length, 8 to 9μ , breadth 6 to 7μ , thickness 5 to 6μ , length of the polar capsule 2 to 3μ and that of polar filament 28 to 35μ .

Habitat. In the gallbladder of *Misgurnus anguillicaudatus* Cantor.

Of the fish examined in September, 1915, 73 per cent. were found to be infected.

Of forms known up to the present time, *Ch. fluviatile* (Thélohan, 1895) seems to be nearest in size and form of the spore and seat of infection to the *Chloromyxum* mentioned. The form and size of the

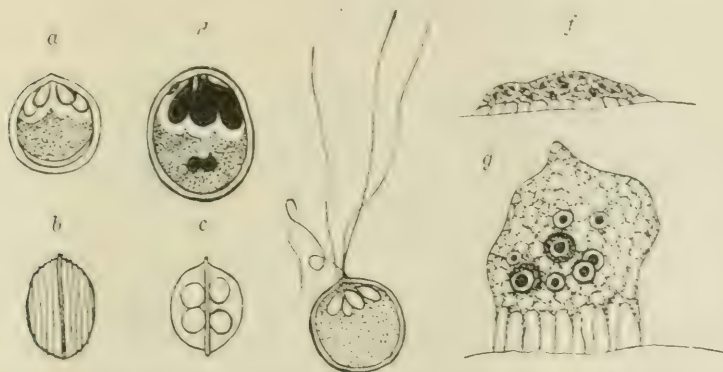


Fig. 3.—*Chloromyxum misgurni* n. sp. a, front view; b-c, side view of the spore in natural condition, $\times 1,750$; d, stained spore, $\times 2,625$; e, spore with the extruded polar filaments, $\times 1,750$; f, g, two young myxosporidia in section, $\times 1,750$.

pseudopodia and the structure of the spore of the type studied differ from that described by Thélohan. Therefore, I propose to name this *Chloromyxum misgurni*.

6. *Chloromyxum fujitai* n. sp.

Vegetative form. Mostly round, sometimes irregular. There is no clear differentiation of the protoplasm. The endoplasm is highly vacuolated, the ectoplasm being hardly visible. The largest one was 40μ in diameter. They float about in the bile, so that in sections of infected gallbladder they are found in the gall apart from the epithelial layer, by which fact we can easily distinguish them from *Chloromyxum anguillicaudati*, even when both forms occur in the same gallbladder. Disporous and polysporous, with up to eight spores.

Spore. Circular in general; often attenuated at the anterior end. Shell bivalve; the line of junction not being straight, but very thick.

The shell has peculiar thick ridges running longitudinally on the surface. Near the anterior end of the spore two small circular markings are clearly visible, in preparations well stained with Heidenhain's iron hematoxylin, one on either side of the line of junction, from which the markings recede on both valves. These two circular markings are not the exits for polar filaments, because it is clearly shown in preparations stained with Giemsa's solution that the four polar capsules have their independent exits. The form of the spore takes different aspects by the presence of the characteristic markings resembling partially those of *Hoferia cyprini* (Doflein, 1898) and *Chloromyxum koi* (Fujita, 1913). In optical cross-section, the spore represents an outline quite like a cog-wheel with twenty to twenty-two ridges, including the widest ridges, which mark the line of junction of the shell. The

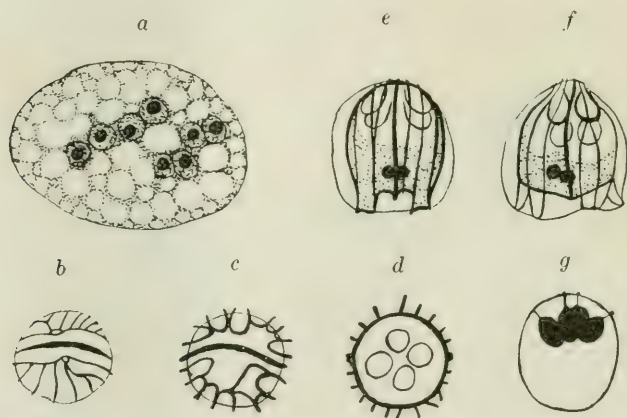


Fig. 4.—*Chloromyxum fujitai* n. sp. *a*, young vegetative form, $\times 1,750$; *b*, *c*, anterior (*b*) and posterior (*c*) view of a stained spore, $\times 1,750$; *d*, optical cross section of the same spore as the above, $\times 1,750$; *e*, *f*, side views of stained spores, $\times 1,750$; *g*, side view of stained (Giemsa) spore, $\times 1,750$.

thickness of the ridges varies regularly. The thickest ones are located where a plane perpendicular to that of junction cuts the shell longitudinally, others decreasing in thickness as they approach the line of junction. Four polar capsules occupy the anterior half of the spore. The sporoplasm contains two nuclei of almost equal size. The dimensions are: length 10 to 12 μ , breadth 8 to 10 μ , length of polar capsule 2 to 3 μ , and that of the polar filament 23 to 30 μ .

Habitat. In the contents of the gallbladder of *Misgurnus anguillicaudatus*. The occurrence is much rarer than that of the former one, showing about 5 per cent. in September, 1915.

Of all descriptions from this genus that of Fujita (1913) alone described similar markings of the spore of *Chloromyxum koi* from the gallbladder of the carp. However, we find a great difference

in form and in the number of ridges. There is also a great difference between the size and structure of the spore, and in the number of the spores found in a vegetative form. So I think this species is a new one. In honor of Dr. T. Fujita, who was the first to study myxosporidia in Japan and to discover the spore of this type, I give the name *Chloromyxum fujitai*.

Multiple infection of the above-mentioned four species takes place very often.

Concerning the pathological effects, I have but little to report. No visible external change could be noticed in any of the infected fishes. But, as in the case of *Acheilognathus*, we found often that the gall-duct had been filled up with a great number of the *Zschokkella* (Fig. 1), therefore it is certain that the secretion of the bile into the duodenum must be greatly disturbed. Such is the case with the highly infected loach, the gallbladder of which has an opaque appearance. It is, however, difficult at present to state the real effects of the parasite upon the host.

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NOTES ON TWO FREE-LIVING LARVAL TREMATODES FROM NORTH AMERICA*

HENRY B. WARD

The life history of parasitic worms has always been a subject of especial interest and no part of it commands more careful attention than that which deals with the brief stages of free existence, for here is the point at which the organism effects its transfer from one host to another. It is only in the few highly specialized types that the parasitic habit endures in unbroken succession from host to host and the parasite is transferred by the agency of the organism in which it is living. This is the case with the *Plasmodium malariae* and all other parasites transferred by a blood sucking host to a new environment in which usually if not always a new generation is developed. It is also the case with the trichina and other encysted parasites which are acquired by a new host thru its carnivorous habit and which develop into a new form or stage of the life history in this new host rather than as a new generation.

In the simpler cases, however, the parasite abandons at intervals the parasitic mode of life and adopts a free-living habit for a stage of its existence or for a generation that alternates with the parasitic type. The free-living generation or stage is of especial interest because it affords the opportunity for the infection of a new host and also furnishes a point of attack in the life cycle which is vulnerable so that the readjustment of environmental conditions may block the transfer and prevent the infection. Such a readjustment may result from the natural operation of external forces, as when a very dry season eliminates the small ponds or swamps in which the free stage develops and thru which it secures means of transfer to the new host. Or the change may be brought about by the introduction of hygienic regulations that are drafted to prevent the parasite from reaching its new host, as the installation of a new sewage system may divert the human feces with tapeworm eggs from the lake into which they were formerly discharged and thus prevent infection of the fish host in that lake with the bladder stage of the fish tapeworm (*Dibothriocephalus latus*); consequently the spread of the tapeworm in the human host is checked.

* Contributions from the Zoological Laboratory of the University of Illinois, No. 71.

Attention has been forcibly directed in the past year to the free-living stages of trematodes by the work of Japanese and European investigators on the life history of the Lungfluke (*Paragonimus*) and the Bloodfluke (*Schistosoma*). As a result of these studies it is possible now for the first time to draw a reasonable picture in outline of the development of these forms. Almost nothing has been ascertained concerning the free-living stages of such parasites in North America. A few observations were made years ago by Leidy and recently Cort has published a careful study of some larval trematodes; but together these cover only a few of the many North American species of fluke. The importance of placing on record all data leads me to print here observations made some years ago although they are yet unfortunately incomplete. For the satisfactory interpretation of these observations a preliminary statement may be made here regarding the process of development as found in the trematode.

Two free-living stages recur in the development of most flukes. From the egg develops a small ciliated larva, designated a miracidium by Braun, which is evidently dependent upon water for its distribution. It remains within the shell until it has reached its full development; thereafter contact with the water is sufficient to open the shell and bring about the escape of the embryo. Active migration through the water permits it to reach and infect the secondary host, a mollusk. It is a somewhat striking fact that in spite of the constant and abundant production of eggs and embryos, no records that I have found note the occurrence of such embryos in plankton or other fresh-water collections. The absence of records can not be attributed to the small size of the miracidia since other even more minute objects fall constantly within the ken of the microscopist engaged in the study of fresh-water organisms, and some that are apparently very difficult to detect have been studied and described in detail. It may be due to the extreme delicacy of the larvae which are thereby readily subject to accidental destruction. Certainly they go to pieces almost as soon as they are collected.

The second free-living stage in the life history of the trematode comes when the cycle of development within the mollusk is completed and the transfer to the adult host takes place. This transfer occurs in the cercaria stage and of course may be direct if the mollusk is eaten by a suitable host. Yet one may safely infer that this is not the usual method since most cercariae are so well adapted to a free aquatic existence. The ordinary cercaria possesses a well developed swimming organ in the tail which characterizes this stage and is cast off when the larva reaches a new host or a place of encystment, as the case may be.

This swimming tail is reduced in a few types and absent only very infrequently.

In other cases the tail is not only present but powerful and displays various modifications, such as bristles, folds, branches, lateral membranes, etc., that increase its functional value. No one who watches living cercariae in the laboratory under experimental conditions can doubt that they are robust swimmers and naturally depend on that method for their transfer from the mollusk to the final host. When infected snails are kept in an aquarium jar on the laboratory table, the cercariae swarm out voluntarily at certain times in great numbers and in many cases can be seen with the unaided eye swimming actively about in the water. They do not confine themselves to the sides or bottom of the vessel but seem to seek equally the open water and in general to conduct themselves under such circumstances like other plankton organisms: protozoa, rotifers, and entomostraca in the same aquarium.

These cercariae are large objects among the microscopic aquatic organisms; they are produced in great abundance and infected mollusks are also abundant and widely distributed. And yet there are almost no records of the occurrence of cercariae in the voluminous reports on fresh water plankton and aquatic life. I am at a loss myself to explain this condition. I have seen them many times in fresh water collections, but only when the material was examined very soon after it was taken in the net. Usually the number of specimens secured was too limited to permit of satisfactorily determining the structure and relationships of the form. In two cases, however, the cercaria was so peculiar as to justify this record of its occurrence even though the description is incomplete in certain respects.

CERCARIA ANCHOROIDES *nov. spec.*

The first species to which attention is called was abundant at Lake St. Clair in 1893 and was described very briefly in a preliminary report (Ward, 1894), as follows:

In the tow was found but one helminth, a form which challenged attention the first of our stay. It is a free-swimming Cercaria, closely allied to *C. mirabilis* Braun, having a prominent tail terminating in two flat blades at right angles to the main body. The distome is enclosed within the tail of the Cercaria which has then more or less the appearance of an anchor with wide flukes. From one to four of these were taken from both top and bottom tow every day from July 27 to August 5 when it suddenly ceased to be found. Efforts to find the intermediate and primary host were alike unsuccessful. This form differs consid-

erably in size from those described by R. R. Wright and M. Braun and is probably a new species.

The cercaria attracted attention by its very active movements, which were so peculiar that it could be readily picked out from other material in a glass dish. While found in collections from the surface as well as in those taken by a runner net and representing thus the fauna near the bottom, yet it was three times as frequent in the latter as in the former. This probably indicates that the cercariae were discharged from snails or other mollusks living on the bottom in the region where the collections were made.

Viewed under a lens the organism appeared as a minute object shaped like a hammer or anchor and swam through the water by a succession of violent jerks. The body moved with the flukes of the anchor in advance and propelled itself by throwing the flukes alternately right and left. In this movement the flexure occurred in the stem of the anchor about one third of the distance from the flukes to the head, and the blades did not move separately but maintained constantly the same relation to each other and to the adjacent part of the stem. The motion recalled distinctly the sweep of a double headed paddle as it is passed from the one side of a canoe to the other.

A more careful examination showed that the stem of the anchor was flat and also the flukes which extended from it nearly at right angles but were curved a little near the outer end. The head of the anchor appeared, however, nearly round, being enlarged and enclosing a small object which lay in a clear, fluid-filled chamber at the extreme head end of the stem (Fig. 2).

The stem of the anchor measured about 2 mm. in length and the flukes were each 0.53 to 0.6 mm. long, though on account of the curve their tips were only 0.84 mm. apart. The flukes varied in width from 0.24 to 0.34 mm. and the stem was 0.28 mm. in maximum width but was reduced to 0.2 mm. at the region of transition from the flat base to the rounded head. At its widest part near the outer end this region had increased again to about 0.3 mm. in diameter.

Under the microscope one could readily distinguish that the object in the enlarged end was a small distome. It lay with the oral sucker near the apex of the chamber and with the opposite end turned towards the flukes of the anchor. In no case was it coiled, twisted, or crumpled together in life but lay flat and straight with abundant space in the chamber for it to be extended to full length. When the distome lay flat on the slide, the base of the stem and the flukes of the anchor stood on edge, thus the breadth of the distome was at right angles to the flat surface of the stem and flukes.

The living distome was faint sulphur yellow with a reddish brown intestine; the anchor stem and flukes were dark by transmitted light and white or faint yellow by reflected light.

Under the pressure of the compressor the young distome (Fig. 1) was forced out of the sac in which it was contained. It emerged at the extreme tip where there seemed to be a preformed opening and left wrinkled and collapsed a thin walled sac in which it had been enclosed. The base of the stem and the flukes of the anchor remained entirely unchanged and often moved about actively in the water for some time after the distome had escaped, beating alternately right and left in the same manner as before and moving freely through the open water just like those specimens in which the distome was still enclosed in the sac.

The young distome, freed from the sac in the tail, as the anchor should properly be called, measured in life 0.64 mm. in length by 0.288 mm. in breadth. The oral sucker was sub-terminal or ventral, being separated 0.016 mm. from the extreme anterior tip; its diameter was 0.16 mm. and its orifice measured 0.048×0.064 mm. The ventral sucker though conspicuous was a little smaller than the oral, measuring 0.128 by 0.144 mm. and being separated from the anterior tip by a distance of 0.27 mm.; its orifice measured 0.04 by 0.072 mm. The pharynx was 0.064 mm. in diameter, and in some cases even a little longer.

The intestine was large, broad, wavy in outline, and filled with a dark reddish brown fluid containing numerous highly refractive granules. The main duct of the excretory system extended from the posterior tip to the acetabulum and two longitudinal trunks were conspicuous on the right and left sides of the worm, outside the intestinal crura. They were not straight but much twisted or thrown into short heavy wavy loops from which fine branches extended towards the margin and gave rise to still finer branches. In one specimen a transverse connection was demonstrated just anterior to the acetabulum extending from the median posterior trunk to the left longitudinal trunk. It was also noticed that the right canal in the same worm was certainly larger near the center of the body than near either end. This condition would indicate that a connection existed at the center in this tube also. One would not go far astray in interpreting the median posterior stem as the bladder from the apex of which near the acetabulum branches extended right and left to divide again near the margin on each side of the body into anterior and posterior trunks.

On the ventral surface of the body appeared a transverse slit just 15μ in front of the anterior margin of the acetabulum in the living specimen, or halfway between it and the oral sucker in a contracted alcoholic specimen. While the ducts connected with it were not yet

developed or at least demonstrable there seems little doubt that this represents the genital pore. In the center of the body behind the acetabulum and between the intestinal crura three faint masses were discernible. The two smaller bodies near each other slightly oblique to the axis and furthest posteriad, are probably the testes and a single larger mass between the testes and the acetabulum is no doubt the ovary. These structures were faint, especially the ovary, and the connecting ducts of the genital system were not yet apparent. The three bodies differ slightly in size and location in different specimens.

The sac at the head of the anchor stem had on the outer surface peculiar vacuolated wart-like protuberances. These may have been sensory structures. In the outer wall were also two sets of fibers—probably muscular—of which those in the transverse series are closer together and very regular whereas the longitudinal fibers are neither so abundant nor so regular. The wall of the sac adjacent to the cavity consists of a thin epithelial layer.

In 1885 R. Ramsey Wright found a single specimen of a similar form swimming actively in a fresh water aquarium at Toronto. He published a brief note (Wright, 1885) in which the form was interpreted as a free-swimming sporocyst. Leuckart to whom the specimen was sent with notes and a sketch published (1886) a more extended description with a copy of Wright's drawing. From this account it is easy to determine that the form is very similar to that described above but not identical with it. Both the length which is "nearly 1 mm." (Leuckart) as against 2 mm. in the new species and the form of the tail as well as of the young distome indicate the specific difference of the two types. As appeared later both Wright and Leuckart were in error in the interpretation of this organism which is not in any sense a sporocyst but a true cercaria although with an unusual type of tail. No name has ever been given to this species, which may be designated *Cercaria wrightii* nov. spec. According to the sketch published by Leuckart (1886:102) the distome fills three fifths of the stem of the anchor, the flukes are nearly straight and together two thirds as long as the stem, and the genital organs of the distome form a solid rod-like mass located above the acetabulum but partly preacetabular and partly postacetabular. The approximate measurements of this form taken from the drawing are, total length 0.75, maximum width 0.133, length of flukes 0.533, breadth of flukes 0.1, length of distome 0.45, breadth of distome 0.1, diameter of oral sucker 0.041, of ventral sucker 0.075 mm. Leuckart speaks of the movement of this species as produced by flapping the two wing-like flat fins, evidently depending on the notes of Wright, though the latter in his printed notes did not refer to the way which the animal moved.

Later M. Braun (1891) published an extended account of a similar form which he also found swimming in an aquarium. The material came from Kurland. He was able to show that the form was not a sporocyst but a true cercaria in which the tail was developed to form a receptacle at the anterior end for the body of the young distome. He traced the larva back to *Limnaea palustris*, var. *corvus* and found in the lungs of these snails the numerous sporocysts from which the cercariae had come and in which could still be seen all stages in their development. He named this form *Cercaria mirabilis*, and decided that it was certainly different from Wright's species. From his description which agrees in general with Wright's type and with my own, some items may be cited to indicate the differences between the three.

Cercaria mirabilis Braun is 6 mm. long with leaflike, movable wings, 1.5 mm. long. In resting it lies on the bottom with folded wings. In swimming the wings are moved actively from side to side, making the motion resemble that of mosquito larvae. The young distome was an opaque yellow body, lying bent in a space lined by a smooth membrane. Granules of yellow pigment occur abundantly forming a network of lines in the bulb wings, and stalk, and also massed in the intestinal crura. The acetabulum is larger than the oral sucker. The rudiments of two testes and the ovary lie behind the acetabulum.

Since the early stages in the development of *Cercaria mirabilis*, taken from sporocysts, are split-tailed cercariae, Braun regarded this form as the higher differentiation of that type. He compared it with a number of known species but found nothing that furnished a real parallel. He was also unable to suggest to what adult distome this larval form should be assigned.

One further point deserves additional emphasis, namely that the method of swimming adopted by all three of these forms differs widely from that of a typical cercaria. While there are minor differences in movement as already noted, yet all accounts agree in stating that the forms travel with the wings in advance, trailing behind them the stem at the end of which lies the distome in the chamber. The distome is so oriented in these cercariae that the anterior end hangs down or back and the posterior end is pointed in the direction in which the larva is moving. It will be clear on comparing this with the usual cercaria that the orientation is precisely reversed; instead of being pushed ahead by the tail the distome in this case is pulled along after the tail. This is an extremely interesting case of the reversal of functional activity.

CERCARIA GORGONOCEPHALA *nov. spec.*

The other cercaria came from Lake Erie near Put-in-Bay, Ohio. It was taken in a tow from a depth of 4 fathoms on July 23, 1901. Unfortunately only a single specimen was obtained. Under a dissecting microscope the object resembled a writhing mass of serpents tied together by the tails. The diagrammatic representation (Fig. 3) gives, unfortunately, little idea of the actual appearance this object presented in life. The bunch contained not less than 50 separate stalks fastened firmly together at the base, all in active motion with a spiral twist passing in wave-like progression from the base to the outer end of the stalk (Fig. 5). The base of each stalk carried a bulbous expansion which in some stages of contraction appeared to be sharply cut off from the rest of the stalk but at other times graded into the stalk without any distinct boundary. This basal enlargement was thicker walled than the stalk elsewhere and possessed yellow pigment granules that were not found in other parts. The stalk was very mobile, delicate in texture and provided with two irregular longitudinal stripes of granular brown pigment which terminated just short of the outer tip. This stalk which is the region of marked contractile activity, tapered slightly (Fig. 4) to the extreme outer tip where it bore the body of a young trematode that appeared to be an amphistome. About half of the stalks had already lost their attachments; most of these still twisted and vibrated nearly as actively as those that carried the young helminths; but a few were pale in color, appeared empty like dead algal filaments, and were motionless. At times all the stalks rested quietly for a brief period and then suddenly began to be violently agitated together. During this movement it was clear that the stalk alone was active whereas the worm was snapped too and fro like the lash of a whip.

As the stalk vibrated with a coiling or twisting motion, the worm at the end was turned at every angle and it seemed as if the large posterior sucker was mounted on a stalk or at least protruded distinctly beyond the general surface of the body. The general form of the amphistome was oval with a concave ventral surface and the anterior end rolled slightly ventrad (Fig. 6) so that the oral sucker was partly concealed. The body of the worm was white, opaque and almost entirely immobile. No internal organs could be detected either in the attached worms or in those that had been shaken off and lay free in the dish. The posterior end of the detached amphistome bore a distinct projection which indicated the point at which the stalk had been attached.

A somewhat similar form was discovered by Claus in the Mediterranean in 1880 and recorded by Leuckart (1886: 87). It is referred to

in various other places as a Rattenkönigcercaria. It was carefully described by Pintner (1891) under the name of *Cercaria clausii* given it by Monticelli in 1888. Odhner has more recently (1911) shown this to be the larval form of *Phyllodistomum acceptum* Lóoss. Apart from the peculiar habit that both forms are borne on stalks tied in bunches there is no close similarity between *Cercaria clausii* and *C. gorgonocephala*. The details of structure in the stalk are as unlike as the structure of the two worms.

It is not without some reserve that the species described above is assigned to the amphistomes. The form of the body suggests this connection but the amphistome cercariae thus far known are very different in type. Cort (1915) has described two species from this group very fully and in common with earlier investigators he finds that two eye spots are present in these cercariae. No eye spots were observed in *Cercaria gorgonocephala*. It is possible that the large sucker of this cercaria is in reality not posterior but ventral and that the postacetabular region is yet undeveloped. No definite opinion on the relationships of this form can be given until further material is obtained.

In one minor structural feature this species manifests a striking resemblance to another amphistome cercaria described by Cort. It is clear that the stalk is the homolog of the cercaria tail and as can be readily seen from an inspection of the sketches published herewith this stalk is attached to the young trematode not at the extreme posterior tip so as to form a direct posterior extension of the body, but rather to the dorsal surface just anterior to the end of the body so that the longitudinal axis of the worm when extended lies ventral to that of the stalk. This same relation mentioned by Cort in his text is beautifully illustrated in his sketch of *Cercaria diastropha* (Pl. 3, Fig. 24). The condition exists also in other amphistome cercariae.

One other point deserves especial notice here. While the stalk in this species is in one way an organ of attachment rather than a swimming organ, yet it retains its muscular development and structure relatively unchanged, and in movement vibrates in a manner which suggests to the eye the close similarity to the moving tail of an isolated swimming cercaria. One can then not find a basis for calling this in a real sense a change of function in the organ. The young distome in *Cercaria gorgonocephala* (Fig. 6) is broader and flatter than any yet described in the group of Amphistomata. North American adult amphistomes are only very imperfectly known and I am unwilling at present to venture a conjecture as to the form to which this cercaria belongs.

The two cercariae discussed in this paper manifest an interesting biological similarity. Both differ from the ordinary cercaria in the peculiar development of the tail, which has become so highly specialized

that its original character is not easily discerned. In one case it has become a very efficient organ of locomotion, conspicuous both for its size and its power; in the other case it has given up its swimming function though not its power, and serves to attach the larval worm to others in the group. In both cases the end result is the same: The larva swimming about in the open water forms a most conspicuous object and is readily snapped up by fishes as Braun determined experimentally with *Cercaria mirabilis*. The conspicuous character of both larvae was also shown in their prompt discovery in the tow although they were surrounded by large numbers of other plankton organisms. Their activity and conspicuousness must be helpful in enabling them to reach a suitable host for further development, and such a host will naturally be sought among the fishes of the waters in which the larvae occur.

SUMMARY

A description is given of the structure and activity of two new cercariae of peculiar type captured free in Lake Erie and Lake St. Clair. They are designated *Cercaria anchoroides* nov. spec. and *C. gorgonocephala* nov. spec., and are compared with known European species. Altho such an occurrence must be common, these forms are the first to be taken in open fresh waters.

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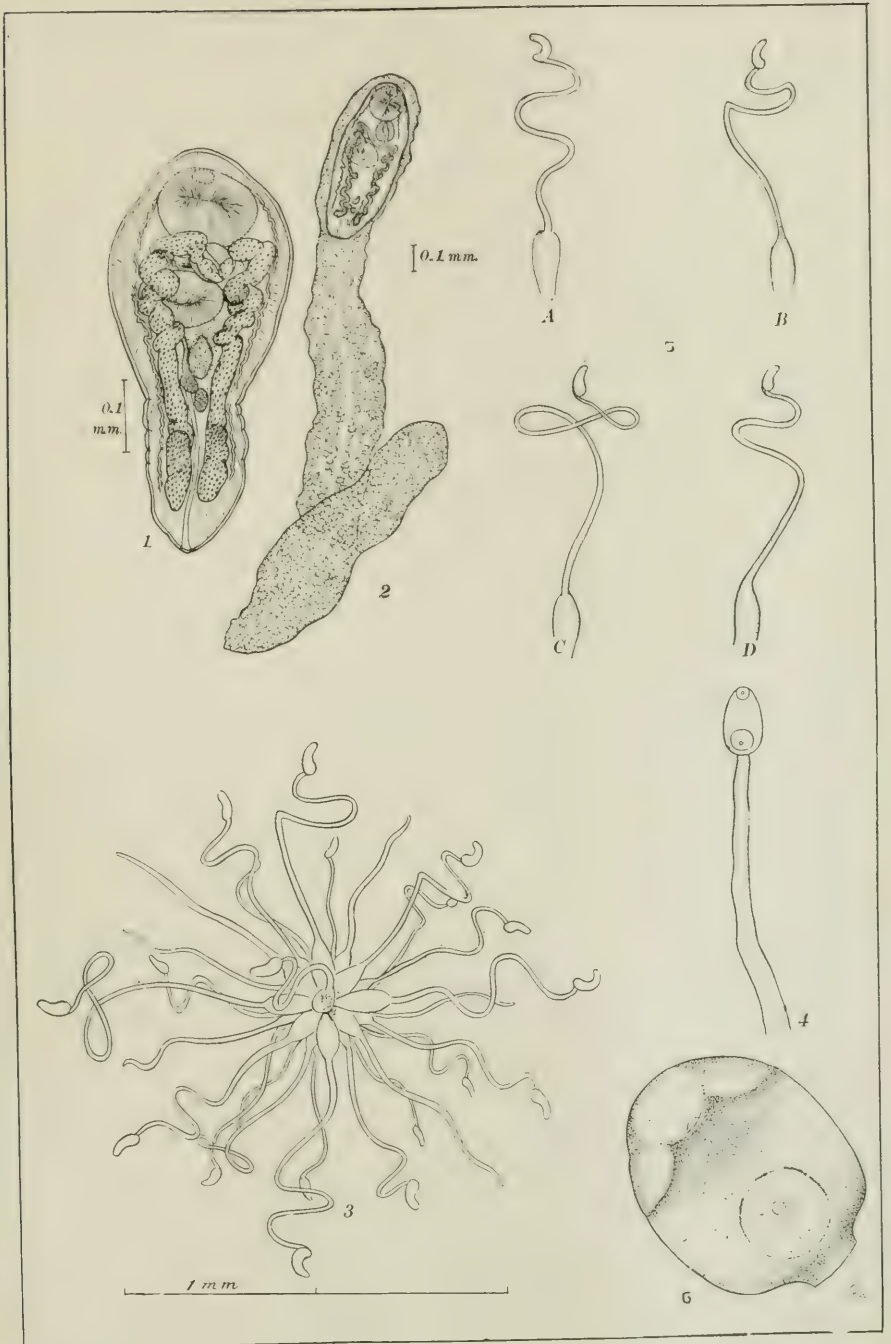
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EXPLANATION OF PLATE

Figs. 1 and 2.—*Cercaria anchoroides*. 1, young distome just set free, $\times 37$; 2, *Cercaria* complete, $\times 105$.

Figs. 3 to 6.—*Cercaria gorgonocephala*. Free hand sketches from life. For details see text.

PLATE



ON THE ANATOMY AND RELATIONSHIPS OF SOME NORTH AMERICAN TREMATODES *

HORACE W. STUNKARD

As the result of an extended study of three families of North American trematodes, Polystomidae, Aspidogastridae and Paramphistomidae, certain points of interest in regard to the structure and classification have been elucidated. Since the publication of the completed work may be delayed, a brief statement of the more important points is presented here in advance of the appearance of the extended paper.

In the latest classification of the monogenetic trematodes, or Heterocotylea as they were termed by Monticelli, Odhner (1912) divided the group into two suborders, Monopisthocotylea in which a "true vagina is present," and Polyopisthocotylea in which a true vagina is wanting and the so-called "ductus vaginalis" is present. After careful study of the female ducts in the Polystomidae, I am able to show that the organ which functions as a vagina is homologous in all monogenetic trematodes and that there can be no division of the group on the basis of differences in this structure. In the complete paper the full evidence is submitted to show that the "true vagina" of the Monopisthocotylea is homologous to the originally single, secondarily paired and subsequently fused vaginae of the Polyopisthocotylea; altho the two suborders of Odhner are nevertheless valid, the essential difference between them is that the genito-intestinal canal is lacking in the former and present in the latter group.

The species that have been included in the genus *Polystoma* show a wider range of structural variation than is usually present in a natural genus. There are marked differences in the character of digestive and reproductive systems and variation exists also in the type of adhesive apparatus. In *P. integerrimum* the ceca are much branched, ramifying thru the body and caudal disc. In *P. alluaudi* the ceca occupy the same location but are merely lobed and have no secondary branches. In *P. bulliense*, according to Johnston (1912), "a diverticulum from the buccal cavity runs backwards, ventral to the pharynx, and for a distance equal to its length forming a median unpaired buccal pocket." In all other known species there is a simple bifurcate intestine, the ceca terminating just anterior to the caudal disc. In two specimens of *P. hassalli*, however, the ceca are connected posteriorly.

* Contributions from the Zoological Laboratory of the University of Illinois under the Direction of Henry B. Ward, No. 72.

The testis is a much branched structure in *P. kachugae*, in *P. integerrimum* it is lobed, and in the other known species it is oval or spherical. In *P. integerrimum* and *P. bulliense* the lateral vaginal swellings are formed by a large number of papillae which are perforated by fine canals, and in all other known species the vaginae are large open funnels and the lateral swellings are reduced or absent. In *P. integerrimum*, *P. bulliense* and *P. alluaudi* there is a long uterus which forms many loops in the intra-cecal area and contains a large number of eggs. In all other known forms, the uterus is situated at the level of the ovary on the opposite side of the body and contains a single large egg or embryo.

The caudal disc bears on its ventral face the chief organs of attachment. These consist of suckers and hooks, the former arranged in pairs, three suckers on either side of the median line. In all previously reported forms except *P. alluaudi*, the anterior suckers are separated by considerable distance giving the disc the shape described by Leidy as cordiform. In the single specimen of *P. alluaudi* described by Beauchamp, both the caudal and cephalic suckers are separated while those of each side are contiguous. In *P. orbiculare* n. sp. each sucker of the disc is separated from the two adjacent to it by uniform distances, making a perfect circle of bothria. In six species studied by the writer these suckers are complicated structures set more or less deeply in the parenchyma of the caudal disc. Their structure, character of insertion and muscular attachments are described in the complete paper. The caudal disc typically bears eighteen hooks. The larval hooks are anchor shaped and are situated six in a row between the anterior suckers, one inside each sucker at the base, and two or four between the posterior suckers. Between the posterior suckers there is also a pair of great hooks several times the size of the larval hooks, and in species in which a single pair of larval hooks is present, there is a third pair of hooks similar in shape to the great hooks and intermediate in size between the great and larval hooks.

In *P. orbiculare* n. sp. neither pair of the great hooks are present, and in *P. opacum* n. sp. there is a single pair of great hooks, very small and poorly developed.

The present study of the polystomes has emphasized the morphological variation and wide geographic distribution represented by the genus. This may mean either that the group is very old and has been subjected to conditions producing wide variation, or that it really lacks generic entity and consists of various heterocotylean forms which have specialized in the direction of an endoparasitic habit and that the morphological resemblance is cenogenetic.

Four new species are added to the genus *Polystoma*, as follows:

POLYSTOMA ORBICULARE *nov. spec.*

Length 2.7 to 3.75 mm.; width 0.9 to 1.2 mm. Caudal disc circular, 0.8 to 1.07 mm. in diameter, bothria arranged symmetrically in a circle. Only hooks present on disc are larval hooks in bases of suckers. Anterior sucker 0.25 to 0.27 mm. in length, 0.37 to 0.42 mm. in width; pharynx spherical 0.24 to 0.28 mm. in diameter; esophagus short. Testis spherical or oval, 0.36 to 0.5 mm. in length, 0.29 to 0.39 mm. in width, near or slightly anterior to middle of body. Genital coronet of 16 hooks equal in length. Ovary lateral, on either side of body, comma-shaped, 0.1 to 0.14 mm. wide by 0.14 to 0.185 mm. long. Vitellaria occupy dorsal and lateral areas from pharynx to caudal disc except in region dorsal to germ glands where they are reduced or absent.

In the urinary bladder of *Pseudemys scripta* from Raleigh, N. C., and of *Chrysemys marginata* from Chicago, Illinois, and Creston, Iowa.

POLYSTOMA OPACUM *nov. spec.*

Length 3.25 to 4 mm.; width 0.8 to 1 mm. Anterior sucker 0.2 to 0.22 mm. long, 0.23 mm. wide; pharynx spherical, 0.3 mm. in diameter; esophagus short. Testis spherical or oval, 0.4 to 0.5 mm. in diameter, slightly anterior to middle of body. Genital coronet of 32 similar hooks. Ovary lateral, comma-shaped or ovoid, 0.16 to 0.2 mm. long, 0.08 to 0.12 mm. wide. Vitellaria strongly developed, extend from pharynx to caudal disc, occupying lateral and dorsal regions of body except area over testis, ovary and uterus.

In esophagus of *Trionyx ferox* and *Malacoclemmys lescurei* from Newton, Texas.

POLYSTOMA MEGACOTYLE *nov. spec.*

Length 2.5 to 2.7 mm.; width 0.71 to 0.78 mm. Caudal disc cordiform; bothria large, overlap. Anterior sucker 0.28 mm. long, 0.35 to 0.42 mm. wide; pharynx 0.35 to 0.38 mm. long, 0.38 to 0.44 mm. wide. Testis near middle of body, 0.28 to 0.33 mm. long, 0.33 to 0.38 mm. wide. Genital coronet contains 36 hooks in one and 42 in another mounted specimen. Ovary broad comma-shaped organ on either side of median line, 0.1 mm. long, 0.075 mm. wide. Vitellaria extend in lateral and dorsal areas of body from pharynx to caudal disc, reduced or absent in small field dorsal to germ glands.

From oral cavity of *Chrysemys marginata*, Creston, Iowa.

POLYSTOMA MICROCOTYLE *nov. spec.*

Length 3 mm.; width 0.78 mm. Caudal disc cordiform; bothria small, separated. Anterior sucker 0.2 mm. long, 0.42 mm. wide; pharynx 0.37 mm. long, 0.4 mm. wide. Testis slightly anterior to

middle of body, 0.36 mm. long, 0.42 mm. wide. Genital coronet of 32 hooks, equal in length. Ovary lateral, 0.075 mm. long, 0.1 mm. wide. Vitellaria well developed, same extent as in *P. megacotyle*.

From oral cavity of *Chrysemys marginata*, Creston, Iowa.

In the family Aspidogastridae the three North American species have been restudied. A detailed comparison of specimens of *Aspidogaster conchicola* with the descriptions of Voeltzkow (1888), Stafford (1896), and other writers confirms former observations and substantiates the statements of Leidy (1851) and subsequent authors that *A. conchicola* occurs in this country. The examination of specimens of *Cotylaspis insignis* and *Cotylaspis cokeri* corrects and supplements former descriptions. Nickerson's (1902) classification of the family is revised and brought to date.

Representatives of three species of paramphistomes have furnished the basis for studies on that family. Two species are from North American turtles and the third is from a duck, *Anas platyrhynchos*. An examination of the literature showed that these forms could not be included in any previously described genera.

A new genus *Alassostoma* is created to contain the two species from turtles. The genus is characterized by the presence of large oral evaginations which open separately from the oral sucker, an esophageal bulb composed of concentric muscle lamellae, germ glands situated near the middle of the body in the median line, both testes anterior to the ovary, vitellaria consisting of small scattered follicles in the lateral and posteriorly in the median area of the body; Laurer's canal opens in the mid-dorsal line, anterior to the opening of the excretory vesicle; cirrus sac and uterus open to the exterior thru a common hermaphroditic duct. *Alassostoma magnum* n. sp. is taken as type of the genus in which is included also *Alassostoma parvum* n. sp.

The genus *Alassostoma* has the type of lymph and excretory systems present in the genus *Schizamphistoma* and designated by Looss (1912) as characters of the subfamily to which that genus belongs. Looss predicted that with the discovery of other genera it would be necessary to create a new subfamily to contain them, and at that time stated the subfamily characters. With the discovery of a second genus so similar to *Schizamphistoma*, the formal erection of the new subfamily is necessary. *Schizamphistoma* Looss was designated as the type genus and the name of the subfamily becomes *Schizamphistominae*. The distinguishing characters of the subfamily are stated by Looss to be two long excretory vesicles which extend singly to the anterior end of the body and a lymph system composed of three canals on either side of the body which extend longitudinally and

break up into many sinuses in the region of the suckers. The subfamily contains the genera *Schizamphistoma*, including also *S. spinulosum* which as indicated by Looss should probably be made the type of a new genus, and the genus *Alassostoma*.

Alassostoma magnum agrees with *Schizamphistoma scleroporium* in general appearance and size, in type of excretory and lymph systems, in character of vitellaria, and in general type of reproductive and alimentary organs; but *A. magnum* has large oral evaginations, which pockets are reduced and do not extend outside the sucker in *S. scleroporium*; further *A. magnum* lacks the preoral sphincter which is present in *S. scleroporium*. In *A. magnum* the uterus and cirrus sac open to the surface thru a common hermaphroditic duct; in *S. scleroporium* they open separately. In *A. magnum* the testes are further porterial and the ovary is situated one fourth to one third of the body length from the posterior end instead of at the level of the anterior margin of the acetabulum as is the case in *S. scleroporium*. In the latter species the testes and ovary are widely separated whereas in *A. magnum* they are relatively close together. *A. magnum* agrees with *S. spinulosum* in the presence of oral evaginations and lack of preoral sphincter, but differs from it in the manner of coiling of the excretory vesicles, in the presence of common hermaphroditic duct, in the character of the vitellaria, as well as in relative positions of the testes and ovary. These morphological data show differences too fundamental to permit the inclusion of *A. magnum* in the same genus with either *S. scleroporium* or *S. spinulosum*.

Alassostoma parvum agrees with *A. magnum* in general morphological features, presence of oral evaginations, lack of preoral sphincter, type of lymph and excretory systems, in character of genital organs and ducts, also in relative position of testes and ovary. *A. parvum* therefore agrees with and differs from *S. scleroporium* and *S. spinulosum* in the same manner as *A. magnum*. That the two American forms are not different developmental stages of the same species is shown by the great difference in size of the worms and relative differences in size of suckers and genital organs. One mounted specimen of *A. magnum* 10 mm. long is not sexually mature while none of the individuals of *A. parvum* are more than 3 mm. in length. *A. magnum* is large with small suckers, whereas *A. parvum* is small with relatively large suckers; and this feature suggested the name *Alassostoma*.

Alassostoma magnum was collected from the large intestine of *Pseudemys troosti* and *P. elegans* from Havana, Illinois, and from *P. elegans* from Chicago, Ill. The specimens of *A. parvum* were found in the cloaca of *Chelydra serpentina* at Urbana, Ill.

The paramphistomes from *Anas platyrhynchos* were collected in Rock County, Nebraska. Unfortunately the fixation of the parasites is such that the excretory and lymph systems can not be traced, altho remnants of both appear in sections. This species closely resembles *Amphistoma lunatum* Diesing. Both are parasites of American ducks, and are the only paramphistomes at present known from avian hosts. They are nearly equal in size, are similar in shape, have a subterminal oral sucker, reproductive and digestive systems that compare closely, and acetabula of the same form, consisting of an anterior portion and a posterior overhanging lip which terminates on either side in a small cone-like projection. The species at hand differs from *A. lunatum* in its smaller oral evaginations, shorter esophagus, and in having oval, lobed testes and ovary instead of spherical germ glands. The acetabulum is nearer the ovary and the vitellaria are entirely extracecal while in *A. lunatum* they extend between the ceca.

Amphistoma lunatum has been placed as an appendix to every classification of the paramphistomes that has ever been attempted. With the discovery of a form so similar that the two must belong together, a new genus is proposed to contain the two species. The peculiar divided condition of the acetabulum suggested the name *Zygocotyle* for the genus. The species at hand, for which I propose the name *Zygocotyle ceratosa*, is designated as type and in the genus is included also the species *Z. lunatum* (Diesing). As diagnostic characters of the genus may be mentioned the subterminal oral sucker, posterior sucker divided or provided with a caudal overhanging lip, absence of cirrus sac, and separate openings for male and female ducts. Others will undoubtedly appear when the character of the excretory and lymph vessels are known. The genus *Zygocotyle* differs from all other known genera of the Paramphistomidae in the ventral position of the oral sucker and the peculiar character of the acetabulum. None of the existing subfamilies will include it fairly, but since the present classification of the Paramphistomidae is somewhat uncertain and the structure of the lymph and excretory systems of this genus is as yet unknown, no further attempt at classification of the group is made at this time.

Types of all the new species described in this paper have been deposited in the Helminthological Collection of the University of Illinois.

SUMMARY

Extended study of North American representatives of the three trematode families, Polystomidae, Aspidogastridae and Paramphistomidae has made possible the first comprehensive treatment in this

country of their structure and classification. Four new species are added to the genus *Polystoma* and three new species of two new genera are added to the Paramphistomidae.

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DAUERCYSTFORMATION OF *TRICHOMONAS* *INTESTINALIS* *

KENNETH M. LYNCH, CHARLESTON, S. C.

Ucke (1908), Bohne and Prowazek (1908), and Bensen (1910), have described encystment of *Trichomonas intestinalis*. Bensen (1910) has also described an encystment for *Trichomonas vaginalis* differing from that of *Trichomonas intestinalis*, and Dobell (1908) reports a dauercyst of *Trichomonas batrachorum*. Alexeieff (1911) disputes the nature of the so-described cyst of *Trichomonas intestinalis*, asserting that it is in reality an ascomyces, a vegetable organism akin to the yeasts, and proposes for it the name *Blastocystis enterocola*. Wenyon (1905) and others uphold Alexeieff's contention, Wenyon calling the organism *Blastocystis hominis*.

The question of encystment of *Trichomonas intestinalis* is an interesting one to me because of the questioned nature of the cyst which has been previously described and because of the prevalence of the parasite in this community. For several years I have been observing *Trichomonas* as a parasite of several locations in the human body and in certain lower animals, and have frequently encountered a form which has proven to be a distinct cyst and not to be confused with that previously described as an encysted *Tr. intestinalis* by Ucke, Bohne and Prowazek, and Bensen, and as *Blastocystis enterocola* by Alexeieff.

At the present I have under observation a man who furnishes this cyst in large numbers and with distinct characteristics. This man is a negro who is in the hospital with chronic endocarditis and who gives no history of dysentery.

In the stool no other protozoon has been found, no cell corresponding to the previously described *Tr. intestinalis* cyst and none of the *Blastocystis* of Alexeieff. There are many active *Trichomonas* conforming to the typical organism, averaging about 8 by 12 micra in size, of elongated pear shape, with constant undulating membrane, three flagella, the stiff spine projecting posteriorly, vacuolated cytoplasm with numerous ingested bacteria, and with nucleus indistinct but showing well in stained preparations. On exposure the active form soon becomes inactive, stationary, and does not develop the irregular ameboid, non-flagellated, undulating form. It is one of the most fixed types I have observed.

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The encysted form is almost as numerous as the active and commonly exhibits a tendency to occur in pairs. It is about three-fourths the size of the active, of a typical pear shape, and has a transparent shell of uniform thickness. The enclosed parasite has a regular ovoid contour and a finely granular grayish appearance. On one side nearer to the small end the nucleus is visible as a refractive granule, and on the other the undulating membrane is seen as a refractive wavy line extending from end to end.

More definite observations of this cyst and its different structures may be made from stained specimens. The technic of staining which I have used is as follows: A thin spread of the feces while still wet is fixed in warm saline alcoholic corrosive sublimate for ten minutes, placed in absolute alcohol ten minutes, iodine solution ten minutes, alcohol ten minutes, washed in distilled water, mordanted in 4% aqueous ferric ammonia alum overnight, stained in 1% alcoholic hematoxylin one day, decolorized in 2% aqueous ferric ammonia alum, counterstained in alcoholic eosin and cleared in carbol-xylol.

In such a preparation the encysted form is more deeply stained than the active. It is about 6 by 8 micra in size and of perfectly symmetrical pear shape. The anterior end projects on a shoulder slightly beyond the general line; the wall is uniformly distinct; and the space between the cyst wall and the body of the parasite is usually distinct and clear, it being usually broader at the anterior end.

The body of the enclosed parasite is of symmetrical, ovoid shape, slightly pointed anteriorly, of dull brick-red color, finely granular and contains no vacuoles or food particles. A fine dark line beginning as a granule in the anterior end runs directly backward to the posterior end. This I take to be the stiffening rib of the undulating membrane because of its close association in origin and termination with that organ. The undulating membrane is distinct as a darker line beginning in close connection with this rib and extending backward along one side of the body in a wavy course to the posterior end, where it curves around the extremity of the parasite and comes to end near this end of the rib. The nucleus is comparatively large, of ovoid form, but lies farther back than in the active parasite and on the side opposite to the undulating membrane. Its usual position is in the posterior part of the anterior half, and between the midline and the body wall. It has a distinct dark-stained rim and a large chromatin mass. This chromatin usually occurs as an irregular black body almost filling the nucleus, but is in some broken into smaller granules and in others distributed around the inner edge of the nuclear rim. In addition to the undulating membrane the cyst usually shows two or three more delicate lines arising in close association with that organ and passing backward over the body for about two thirds of its length. These are

probably flagella. They stain poorly and are not constantly seen, especially in the more faintly stained specimens. The characteristics of the fully formed cyst may be seen in Figure A3 which is an off-hand drawing.

In addition to this fully developed cyst which predominates, there are young cysts and forms of apparent pre-encystment. There is a form which is smaller than the active parasite, shorter and more blunt, with cytoplasm somewhat reticulated, but showing no vacuoles and no bacteria or other ingested materials, and with nucleus more distinct and with larger amounts of chromatin. This I take to be a pre-encysted stage. Figure A1 appears to be a young cyst before the wall has reached full formation. Its shape is typical; the body wall is thick; and the internal organs appear as in the fully encysted. A further stage appears to be the form in which there is a space between the shell and parasite only at the anterior end, these parts being in immediate contact around the remainder of the body, see A2.



Fig. A1, 2, and 3.—Drawings of different stages of dauercysts of *Trichomonas intestinalis* as seen in specimens stained in hematoxylin.

Further development suggestive of multiplication I have not seen in these cysts; and the preservation of the undulating membrane and flagella, together with the single nucleus, indicate that the process is not for reproduction but simply for resistance.

DISCUSSION

According to these observations the formation of a resistance cyst plays a part in the life of *Trichomonas intestinalis* as occurs with other intestinal protozoa, and I believe is the stage in which the parasite may be transmitted. That infection by the contracted but non-encysted parasite occurs I am not prepared to dispute, and it seems probable to me that the contracted form may be a pre-encystment stage. Reasoning by analogy leads one to believe that infection through the stomach by means of the non-encysted is not probable, and certain observations which I have made of the purely active *Trichomonas* from the vagina also rule against such a manner of infection.

I have previously reported (1915) a case of vaginal and mouth infection by *Trichomonas* which did not infect the intestine, this being determined by repeated examination after purging. I have since had a similar case of trichomoniasis of the vagina and mouth; and again by repeated antemortem and also postmortem examinations failed to find the intestine infected. In these cases the parasites seemed identical in the two situations, and there was never any but the pure fixed type of active form. There were enormous numbers in the mouth for

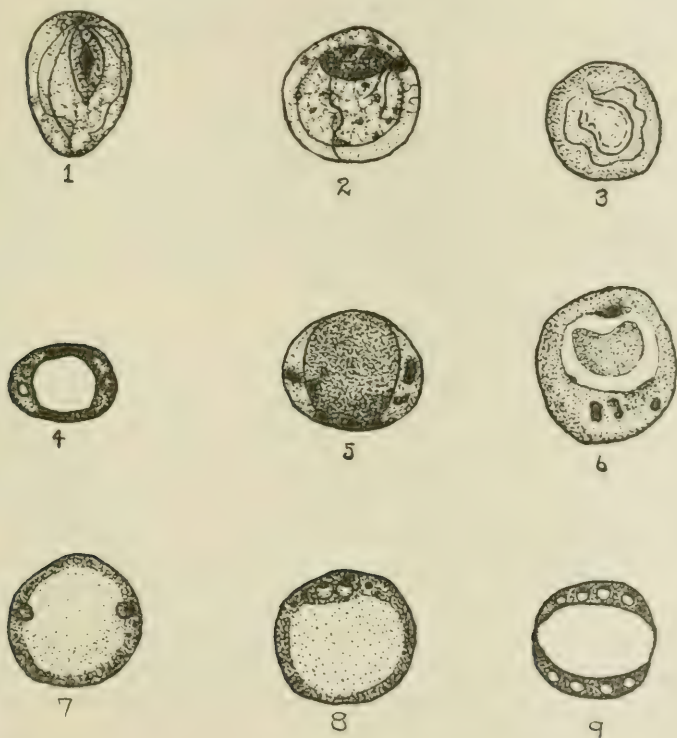


Fig. B1, Dauercyst of *Tr. batrachorum* by Dobell; 2, Encysted *Tr. vaginalis* by Bensen; 3, Cyst of *Tr. intestinalis* by Wenyon; 4, Encysted *Tr. intestinalis* by Ucke; 5, Encysted *Tr. intestinalis* by Bohne and Prowazek; 6, Encysted *Tr. intestinalis* by Bensen; 7 and 8, *Blastocystis enterocola* by Alexeieff; 9, *Blastocystis hominis* by Wenyon.

considerable periods of time; and if the active *Trichomonas* is capable of transmission through the stomach to the intestine in man, the swallowing of these organisms should have produced an intestinal infection in these women.

It is not my purpose at this time to enter into a discussion as to the nature of the previously described encysted *Tr. intestinalis* which has been called *Blastocystis* by Alexeieff; but from extensive observa-

tion of both organisms, both associated and occurring separately, I am in accord with the view that it is not a *Trichomonas* cyst.

In order that the lack of resemblance of this cell to the cyst here described may be seen I have included copies of figures by Ucke, Bohne and Prowazek, Bensen, Alexeieff, and Wenyon. Figures B5 and 6 are from a hematoxylin-stained specimen. Hence the main difference in appearance to Figures B4, 7, 8, and 9, which are representations of the unstained cell, since the internal part of the cell, which is often transparent in the fresh specimen, stains rather deeply and the nuclei are more distinct in the stained. Figures B4, 5, and 6 are illustrations of the so-called *Trichomonas intestinalis* cysts of Ucke, Bohne and Prowazek, and Bensen. Figures B7, 8, and 9 are illustrations of *Blastocystis* by Alexeieff and Wenyon. There is seen to be no point of similarity between the *Trichomonas* cyst of Ucke, Bohne and Prowazek or Bensen and that here described and pictured in Figure A; whereas, barring differences in preparation and in stage of the organism, the *Blastocystis* of Alexeieff and Wenyon and the *Trichomonas* cyst of Ucke, Bohne and Prowazek and of Bensen are apparently one and the same organism.

There is however some resemblance between this cyst and Bensen's encysted *Trichomonas vaginalis* (Figure B2) and Dobell's cyst of *Trichomonas batrachorum* (Figure B1), which I have taken the liberty of copying for comparison. In the last two the flagella are preserved until the cyst is formed, but lost afterwards; while the typical shape of the *Trichomonas intestinalis* cyst is not observed, and the undulating membrane and axostyle are not seen. The nucleus is also different, in that in *Tr. intestinalis* it is more rounded and placed more posteriorly, while in both *Tr. batrachorum* and *Tr. vaginalis* it is forward, larger and spindle shaped. Then in the case of *Tr. vaginalis* Bensen has described and illustrated further development of the cyst for multiplication, while further development of the cyst here described has not been seen.

CONCLUSION

Accordingly, therefore, the formation of a resistance cyst does play a part in the life of *Trichomonas intestinalis*, and this cyst bears no relationship to the cell which has been previously described as an encysted *Tr. intestinalis* by Ucke and others, and as *Blastocystis enterocola* by Alexeieff, and differs essentially from Dobell's dauer-cyst of *Trichomonas batrachorum* and Bensen's encysted *Tr. vaginalis*.

SUMMARY

Encystment of *Trichomonas intestinalis* has been investigated for many years. That which has been previously described has not stood

the test of investigation. The morphology of the cyst here described identifies it with the parasite from which it arises. It is not to be confused with any cell occurring in the intestine and feces. Whether this cyst releases more than one organism or whether *Trichomonas intestinalis* has a multiplication cyst remains an unanswered question.

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NOTES ON TWO CESTODES FROM THE SPOTTED STING-RAY

EDWIN LINTON

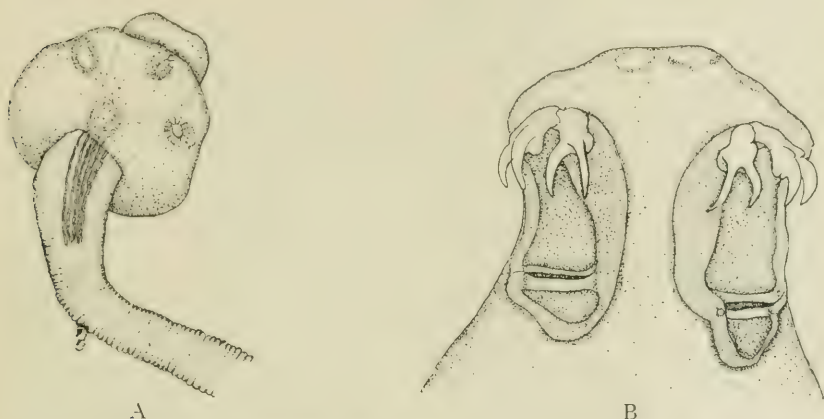
A single specimen of a species of cestode found in the spiral valve of a cow-nosed ray (*Rhinoptera bonasus*) at Woods Hole, July 29, 1887, was made the type of a new genus and species (*Tylocephalum pingue*). No other examples of this genus have been found at Woods Hole, but on June 30, 1908, at the Tortugas laboratory, I obtained two specimens of a cestode from the spotted sting-ray (*Actobatis narinari*) which are to be referred to the genus *Tylocephalum*. The specimen from the cow-nosed ray was a less mature strobile than those from the spotted ray; a comparison of the genitalia, therefore, cannot be made. There appears to be enough difference, however, in other particulars to justify referring the Tortugas specimens to a new species. While both hosts belong to the family of eagle-rays, there is enough difference between them in the way of geographical range and generic features to make it unlikely that the same species of cestodes should be found in each.

TYLOCEPHALUM MARSUPIUM *nov. spec.*

Scolex: The relatively large, muscular portion (myzorhynchus) is subglobular, its length in a living specimen 0.16 and breadth 0.21 mm.; bothria united into a subglobular disc with four auxiliary acetabula, length of disc 0.30, breadth 0.69 mm. The constriction noted in the Woods Hole specimen not present. As in the case of the specimen from the cow-nosed ray, the scoleces were rather firmly fastened to the mucous membrane of the spiral valve. One of the worms was fixed without detaching it, and was sectioned together with a small piece of the intestinal wall. The sections show that the myzorhynchus alone had entered the mucous membrane.

Strobile: The segments begin nearer the scolex than they do in *T. pingue*. Just behind the scolex, where the breadth was 0.16, the strobile was crossed by crowded lines. One-half millimeter back of the scolex the well-defined segments were 0.014 mm. in length and 0.18 mm. in breadth. Three millimeters back the length of the segments is about 0.05 and the breadth 0.24; ten millimeters back the length is 0.12, the breadth 0.28; twenty millimeters back the length is 0.28, the breadth 0.24; thirty millimeters back the length is 0.46, the breadth 0.28; forty millimeters back of the scolex the length is 0.56, the breadth 0.38 mm. The last segments are somewhat variable in their dimensions, but are about one millimeter in length and 0.5 mm.

in greatest breadth. They are vase-shaped, constricted at the anterior end, swelling out to the maximum breadth behind the middle, slightly constricted near the posterior end with a moderately projecting posterior margin. One proglottis had the following dimensions: Length 0.84; breadth, anterior 0.21, maximum 0.56, posterior 0.39 mm. The strobile is especially distinguished by the strongly developed longitudinal muscles. The longitudinal muscles are disposed in radial bundles near the scolex (Fig. 1), but farther back lie in a well-defined zone (Fig. 2). In segments in which the genitalia have become differentiated this zone of muscle bundles coincides in position with the vitellaria (Fig. 4).



Text Fig. A.—*Tylocephalum marsupium*. View of scolex in life, somewhat flattened and seen from behind. Breadth of scolex 0.7 mm.

Text Fig. B.—*Onchobothrium tortum*. Side view of scolex; balsam. Diameter at base of hooks 0.64 mm.

Genitalia: The general plan of arrangement of the genitalia is shown in Figure 7. The vitellaria are peripheral and consist of rather finely granular masses lying between and also centrally to the muscle bundles. The testes are in the median region. In the younger proglottids they occupy most of the interior, but as the proglottids mature they give way to the seminal receptacle and ovary. The cirrus-pouch is relatively small and oval, opening near the margin not far from the middle of the length. The vagina opens into the genital cloaca, passes along one side of the cirrus pouch, becomes more or less convoluted and expands into a capacious seminal receptacle. This was filled with spermatozoa in all the later proglottids. The ovary is lobed and is situated at the posterior end of the proglottis.

The uterus was still rudimentary even in the mature proglottids. In a section a small cluster of minute bodies was seen. They lay in the lumen of the uterus, were yellowish brown, and about 0.010 by 0.007 mm. in the two principal diameters.

ONCHOBOTHRUM TORTUM *nov. spec.*

Ten specimens of this form were obtained from a spotted sting-ray (*Aetobatis narinari*), June 30, 1908. The scolices were imbedded in the intestinal wall and had caused some ulceration. One of the worms, straightened out on a glass plate in sea water, measured 220 mm. in length. Anterior end sub-cylindrical, with a tendency to coil spirally; color dark ashy-gray. Scolex long-clavate, armed with four pairs of short, sharp, two-pronged hooks. Each pair of hooks situated at the anterior end of one of the four bothria. The latter are oblong, trough-shaped, with two costæ near the posterior end. Behind the scolex the body is at first sub-cylindrical and crossed by fine, closely crowded lines for a considerable distance. The segments outlined by these transverse lines remain closely crowded, while the adult proglottids begin rather abruptly. The average length of the first 12 adult proglottids was 0.8 mm., the breadth being about the same or slightly greater. The diameter of the sub-cylindrical portion of the strobile was about 1.5 mm. The scolex and anterior portion of the strobile are much thicker than the adult proglottids. Diameter of scolex, in alcohol, anterior 0.85, middle 0.77; diameter of neck, a short distance back of the scolex, 1.4 mm. Dimensions of one of the posterior proglottids: life, length 1.47; breadth, anterior 0.5; middle 0.8, posterior 0.6 mm. Dimensions of scolex mounted in balsam: length 0.97; breadth, at base of hooks, 0.97, behind hooks, 0.81; breadth of neck, a short distance behind the scolex, 1.27 mm. In the mounted specimen the neck is seen to be traversed by strong longitudinal muscle bundles which are closely crowded together, each bundle about 0.06 mm. in diameter. About 16 bundles were counted near the head; farther back they are divided into a larger number of smaller bundles. Two spiral vessels show distinctly in the mounted specimen. The strobile narrows as the proglottids become distinct. In the specimen which measured 220 mm. there were distinct and well-formed segments on the last 150 mm. The maturing segments were at first much broader than long, then squarish, then longer than broad, the last ones three times as long as broad. The posterior margins of the proglottids project slightly and have crenulate borders. One of the posterior proglottids of a mounted strobile has the following dimensions: length 1.86; breadth, anterior 0.36, constriction near anterior end 0.25, middle 0.40, posterior margin 0.54 mm. The genital apertures are marginal at about the middle of the length. They are irregularly alternate. No ova were seen.

The general plan of arrangement of the genitalia is shown in Figure 8. The cirrus is armed with slender, bristle-like spines; a few folds of the vas deferens are included in the oval cirrus-pouch at its

medial end. The voluminous folds of the vas deferens form the seminal vesicle and occupy the median third of the anterior half of the proglottis. The testes are situated in the anterior half of the proglottis, and occupy the median region on each side of the vas deferens. On the marginal sides of the testes are the vitelline glands which extend along each marginal border of the entire length of the proglottis, being interrupted only at the point where the cirrus pouch and the accompanying vagina approach the genital aperture. The uterus was represented by a tubular structure lying along the median line near one of the lateral faces of the proglottis, and extending from nearly one end of the proglottis to the other. The ovary is a lobed organ and fills all the space between the marginal vitellaria behind the cirrus pouch. The vagina opens at the genital pore immediately in front of the cirrus and lies alongside the anterior border of the cirrus pouch. At this point it is thick-walled and glandular. It becomes tubular at about the level of the median end of the pouch and passes along the median line beneath the uterus to about the middle of the ovary. The relative positions of vagina and uterus are shown in Figure 9, which is sketched from a transverse section of a maturing segment at a level which passes very near the genital aperture, shows a portion of the vagina near the margin, cuts into some folds of the vas deferens, and passes thru the vagina again near the middle of the segment, where it lies on the medial side of the uterus. The section also catches a few of the anterior lobes of the ovary. In this section the characteristic longitudinal muscles are seen as an inner circle of larger and an outer circle of smaller bundles. The lateral vitellaria and the median testes flanking the folds of the seminal vesicle are also shown.

SUMMARY

Two new species of cestodes, of the genera *Tylocephalum* and *Onchobothrium*, respectively, are described in this paper. One of them, *T. marsupium*, is the first cestode of this genus to be recorded since the genus was established in 1887. Thus far representatives of this genus have been found only in the eagle rays.

Altho the two genera belong to quite different families, they possess an interesting feature in common in the strongly fasciculated longitudinal muscle layers. Both species were fastened to the mucous membrane of the spiral valve which, at the point of attachment of the onchobothria, was somewhat ulcerated.

EXPLANATION OF PLATE

Fig. 1.—*Tylocephalum marsupium*. Transverse section of neck. Diameter 0.22 mm.

Fig. 2.—*Tylocephalum marsupium*. Transverse section of early proglottis, showing rudiment of genitalia and peripherally arranged longitudinal muscle bundles. Greater diameter 0.65 mm.

Fig. 3.—*Onchobothrium tortum*. Transverse section of neck, showing longitudinal muscle bundles and vessels of the vascular system. Longer diameter of section 1.12 mm.

Fig. 4.—*Tylocephalum marsupium*. Transverse section of mature proglottis in front of cirrus bulb; longer diameter 0.45 mm.

Fig. 5.—*Onchobothrium tortum*. Longitudinal view of neck showing muscle bundles. Breadth 1.17 mm.

Fig. 6.—*Onchobothrium tortum*. View of retracted cirrus, and vagina; from longitudinal section.

Fig. 7.—*Tylocephalum marsupium*. Posterior proglottis; outline from life; genitalia partly diagrammatic. Length 0.8 mm.

Fig. 8.—*Onchobothrium tortum*. Posterior proglottis; balsam. Length 1.6 mm.

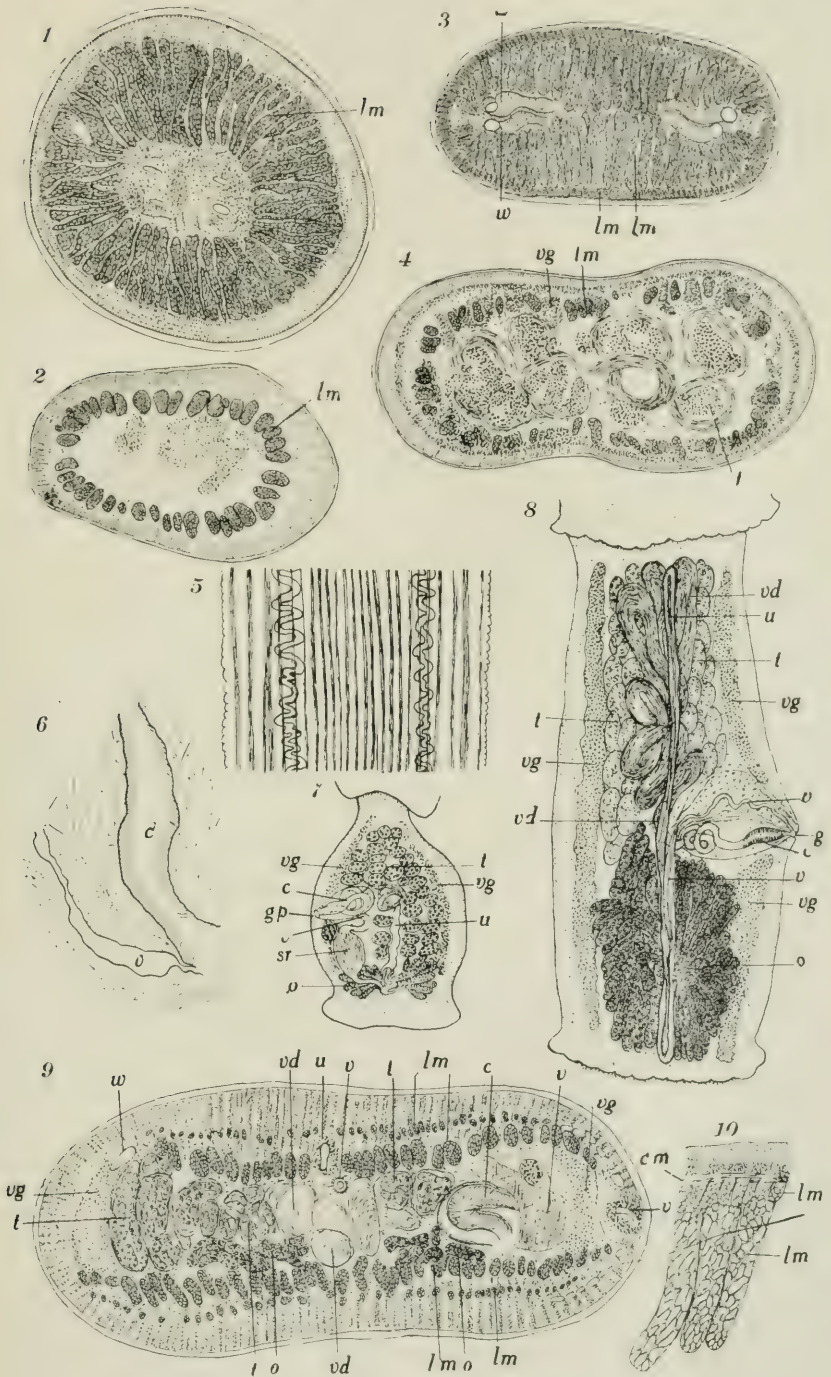
Fig. 9.—*Onchobothrium tortum*. Transverse section of a somewhat younger proglottis than that shown in Figure 8. Longer diameter of section 1.12 mm.

Fig. 10.—*Onchobothrium tortum*. Details of musculature.

ABBREVIATIONS USED

<i>c</i> , retracted cirrus	<i>t</i> , testes
<i>cm</i> , circular muscle layer	<i>u</i> , uterus
<i>gp</i> , genital pore	<i>v</i> , vagina
<i>lm</i> , longitudinal muscle bundles	<i>vd</i> , vas deferens
<i>o</i> , ovary	<i>vg</i> , vitellaria
<i>sr</i> , seminal receptacle	<i>w</i> , longitudinal vessel

PLATE



A CASE OF THE OCCURRENCE OF *ASCARIS TRIQUETRA* SCHRANK IN DOGS *

A. C. WALTON

While working on the spermatogenesis of certain Ascaridae last year, I found that the chromosomes of the ascarids from dogs did not agree with those of the ascarids from the dog as given by Kultschitzky (1888) and by Marcus (1906) either in number, behavior, or the presence of an idiochromosome group. The work of Glaue (1908, 1909, 1910) has shown conclusively that the ascarids of the dog and of the cat are anatomically distinct species, which should be designated respectively as *Ascaris canis* Werner, and *Ascaris felis* Goeze, and not merely varieties of *Ascaris mystax* Zeder. The work of Edwards (1911) on *A. felis* and that of Marcus (1906) on *A. canis* have given us conclusive evidence that these two forms are entirely dissimilar as to the number and the behavior of the chromosomes. From these taxonomic and cytological proofs, the long mooted question of the identity of the two varieties seemed definitely settled; but the apparent contradiction in the gametogenesis of the dog ascarids shown by my discovery seemed to me sufficient to warrant the reopening of the question. If the number and behavior of the chromosomes in *Ascaris canis* were similar to the number and behavior of those in *Ascaris felis*, the two forms might be in fact only sub-species; varying taxonomically owing to their different environments.

The results of my study are contained in a paper now in press, the taxonomic work of which showed that the species with which I was working were the ones recognized by helminthologists as the usual inhabitants of the intestine of the dog and the cat, respectively. My work on the gametogenesis of *A. felis* agreed with that of Edwards (1911) in showing nine chromosomes as the haploid number, one of which is a member of an X-Y idiochromosome pair.

Marcus (1906) has shown that what he called *A. canis* has ten paired and two unpaired tetrad chromosomes as the diploid number. From his description it seems probable that these two unpaired chromosomes act as members of an X-Y idiochromosome group, but he did not so call them. My work on the commonest parasite of the dog has shown that in the male there are thirty tetrad chromosomes as the diploid number, of which twenty-four are united in pairs, and the

* Contributions from the Zoological Laboratory of the Museum of Comparative Zoology at Harvard College, No. 283.

other six form a heterochromosome group of the X type. The female has thirty-six tetrads (eighteen di-tetrads) as the diploid number.

Private correspondence between Dr. S. I. Kornhauser, of Northwestern University, and Dr. Marcus has shown that the majority of the material upon which the latter based his work was *not* obtained from dogs, but came mostly from other members of the dog family and also from bears.

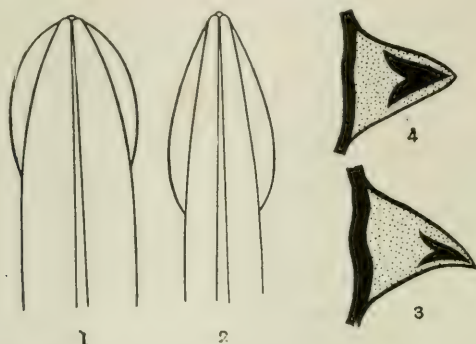


Fig. 1.—Dorsal aspect of the anterior end of *Ascaris triquetra* Schrank ($\times 25$).

Fig. 2.—Same view of *Ascaris canis* Werner ($\times 25$).

Fig. 3.—Cross-section. Posterior aspect of the right wing of *A. triquetra* ($\times 160$).

Fig. 4.—Same for *A. canis* ($\times 160$).

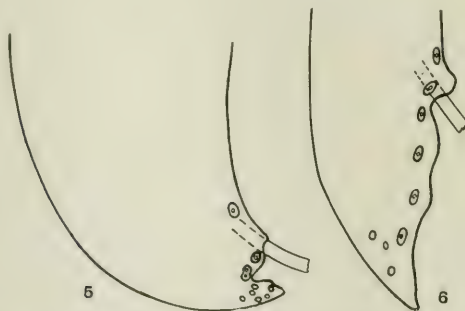


Fig. 5.—Lateral view of the right side of posterior end of male *A. triquetra* ($\times 25$).

Fig. 6.—Same view of male *A. canis* ($\times 25$).

All drawings were made with the aid of a camera lucida.

During the past two years I have been able to examine worms taken from twenty-five dogs, and of the total of two hundred worms thus obtained, all but two have answered taxonomically and cytologically to the type described above as the commonest *Ascaris* in dogs, i. e., *Ascaris canis* Werner. These two exceptional specimens, a male

and a female of the same species, differed considerably in taxonomy from the ordinary type of *Ascaris canis* Werner. The following table compares the main features of the two forms:

	<i>A. canis</i> Werner	<i>A. triquetra</i> Schrank
Length of male.....	120 mm.....	60 mm.
Length of female.....	220 mm.....	100 mm.
Shape of oral wing.....	Lanceolate	Broadly lanceolate
Thickness of oral wing.....	0.17 mm.....	0.18 mm.
Breadth of oral wing.....	0.165 mm.....	0.18 mm.
Length of oral wing.....	2.7 mm.....	1.9 mm.
Chitin rod of wing.....	Long and broad.....	Shorter and narrower
Post-anal papillae.....	7	8
Ventral row.....	4	4 (one double).
Dorsal row.....	3	4 (2 rows, 2 each)
Shape of tail of male....	Slopes gradually to a point	Bends sharply ventrad to a short, blunt end

The comparison of the two species shows that the less common one agrees with *Ascaris triquetra* Schrank, which earlier writers believed to be synonymous with *A. mystax* Zeder and *A. marginata* Rudolphi. Marcus (1906) had identified his *A. canis* with the *A. marginata* studied by Kultschitzky (1888). Cytological examination of the sex cells of this *Ascaris triquetra* Schrank shows that there are twenty tetrad chromosomes, arranged in ten pairs, and also two unpaired tetrads, as the diploid number. This agrees with the facts recorded by Marcus for his material, and I believe, therefore, that the *Ascaris* studied by him was also *Ascaris triquetra* Schrank, known to Kultschitzky as *Ascaris marginata* Rudolphi.

My work, then, has shown that, while *Ascaris canis* Werner is the common parasitic nematode of the dog, *Ascaris triquetra* Schrank may be an inhabitant of the same dog that harbors individuals of the species *A. canis* Werner, though this occurs only rarely. It has also shown that the nematode studied by Marcus (1906) was probably *Ascaris triquetra* Schrank, rather than *Ascaris canis* Werner.

I wish here to express my obligation to Dr. S. I. Kornhauser for his notes and especially to Dr. E. L. Mark for his supervision of the preparation of this paper.

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REVIEWS AND NOTES

The staff of the Research Department at the Severance Union Medical College in Seoul, Korea, of which Dr. Ralph G. Mills is director, has undertaken a review for English readers of current periodicals in Japanese medical literature. This is printed every two months in the *China Medical Journal*, and also circulated separately. The publication is likely to be of great importance to parasitologists because of the activity in Japan at present in the investigation of diseases caused by animal parasites which play a great rôle in that country.

The first (?) number, dated 1916 and recently received, contains a review with illustrations of long articles on the development of the supposed last stage in the life history of *Paragonimus* by Nakagawa, on the first intermediate host of that parasite by the same author, and on an investigation of the Lungfluke in Korea by Kakami, in addition to numerous other items mostly pathological. The reviews are very well written and present valuable material not otherwise accessible to the American investigator. ●

It is with great sorrow that the *JOURNAL* announces the death of one of its collaborators, the distinguished German helminthologist, Max Lühe, Professor at the University of Königsberg, who died of wounds received in the war. The death of Lühe is a great loss to science and the world. His contributions to the literature of parasitology embrace important and extensive studies on Protozoa, Trematoda, Cestoda, Nematoda, and Acanthocephala. It is hoped to print at an early date a biographical sketch of Professor Lühe accompanied by a portrait.

Harvard University has issued the first formal announcement of the School of Tropical Medicine. Courses are open to graduates of recognized medical schools so that the work becomes a part of the Graduate School of Medicine. Dr. Richard P. Strong is Director of the School of Tropical Medicine and in the work announced are courses in protozoology, helminthology, and tropical entomology.

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Number 2

THE EFFECTS OF RADIATION ON THE DEVELOPMENT OF *TRICHINELLA SPIRALIS*

WITH RESPECT TO ITS APPLICATION TO THE TREATMENT OF
OTHER PARASITIC DISEASES

E. E. TYZZER AND JAMES A. HONEIJ

Since radium has been shown by biological experiment to have a pronounced effect on the development of the germ cells of various species, the possibility of its utilization in the destruction or even in the emasculation of certain parasites for which there is at present no efficient remedy appears worthy of consideration. It was thought that radium might be employed to advantage in the treatment of cases of schistosomiasis in which the bladder is involved, several of which have been under the authors' observation for a considerable period of time. Although this condition is of common occurrence in certain parts of the world and although it is frequently attended with serious complications, up to the present time no successful form of treatment has been discovered. Since the inflammation in this disease is produced by the presence of the ova in the tissues and since the worms from which the latter are derived, are situated in close proximity to the mucous surface of the bladder, this mode of attack seemed to be especially appropriate. It did not appear justifiable, however, to undertake the treatment of human cases without a certain amount of preliminary experimentation.

While the use of Roentgen ray for the treatment of schistosomiasis has been suggested,¹ there appears to be an advantage in the use of radium or its emanation in this disease, for the bladder wall in which the worms are situated may be radiated directly from its inner surface and rays of shorter wave length may be utilized than is possible with the Roentgen rays. According to Packard (quoted by Abbe, 1914) the beta rays are more effective than the gamma rays in retarding the development of certain species.

1. The advisability of employing the x-ray therapeutically in this disease was discussed by Doctor R. Gonzales Rincones of Venezuela at the recent Pan-American Scientific Congress at Washington.

In the following experiments radium emanation was employed as follows:

1. To radiate from the outside the abdomen of rats previously fed with the cysts of *Trichinella spiralis*.
2. To radiate muscle containing encysted larvae of this parasite.
3. To radiate the worms directly during their development in the intestine by feeding minute glass tubes containing radium emanation.² Radiation was accomplished in several ways and further details will be presented with the account of each experiment.

Technic.—The effects of radiation on the parasite were judged by either the failure of the larvae to develop in the intestine of rats and mice or by abnormalities in their development. It was thus important to determine the number of worms present in the intestine and also to note any retardation in their differentiation or growth. In order to count the worms, the intestine of the animal to which the larvae had been fed was cut into pieces of from 3 to 4 cm. in length. These were each placed on an ordinary microscopic slide, opened with fine scissors, and by fixing one end of the piece with tweezers the mucosa was completely stripped from the muscular wall by several light sweeping strokes with the edge of a scalpel. The material obtained, i. e., mucosa and softer portions of the intestinal contents, was spread slightly, and then pressed gently beneath a large 22 by 40 mm. cover glass. With a microscope equipped with a mechanical stage, all the worms in such preparations may be readily observed and counted. Since the material is flattened into a thin film the anatomy of the worms is clearly apparent so that an accurate enumeration of the sexes may readily be made. In the earlier experiments equal amounts of muscle taken from corresponding portions of the body of an infected animal were used for infecting the radiated and the control series, respectively. Since this procedure furnished only approximately equal dosage in the later experiments with mice, the cysts contained in strips of diaphragm were counted and an equal number fed to each of a series of animals.

Observations made during the course of this study failed to confirm certain statements, which occur quite generally in standard works, concerning the anatomical distribution of *Trichinella spiralis*, the ratio of the sexes, and the span of life of the adult male and female of this species.

Distribution in Rats and Mice.—*Trichinellae* are said to mature in the duodenum and jejunum and it might be inferred that the adults are confined to the first portion of the intestine. In the course of the

2. The authors are indebted to Doctor William Duane for collecting and measuring the radium emanation used and also for suggestions as to dosage, filtration, etc.

following experiments the worms were comparatively rarely present in the first portion of the small intestine of rats and mice, but were found in great numbers throughout the remainder. They not infrequently occur also in the cecum and colon of mice and occasionally in the large intestine of rats. It appears probable that the small size of these host species may account for the presence of the worms in the large intestine since no great extent of gut would have to be traversed before reaching the cecum.

Sex Ratio.—According to Stäubli (1909), great discrepancies with respect to this point are found in the statements of different authors. Thus Leuckart reports the females as greatly in excess of the males, in one instance in a 10:1 or 20:1, and in another instance in a 6:1 ratio. Zenker calls attention to the difficulty in finding the males on account of their smaller size. Askanazy, on the other hand, finds the males greatly in excess in the intestinal contents, but this was thought to be due to the fact that the females burrow into the mucosa, while the males remain free. Ostertag claims that the males and females are originally present in equal numbers, but that the former after copulation diminish in number, so that after 10 to 14 days only females are present. Both sexes were observed by Pagenstecher 56 days after ingestion. Stäubli notes great variation in the sex ratio in different cases with respect not only to the mature adults, but also to the encysted larvae the sex of which he is able to distinguish. He is unable to account for this lack of uniformity in the relative number of the sexes.

In order to avoid error in estimating the relative number of males and females it is important to examine the material in such a way that none will be overlooked. The males, on account of their smaller size, are not so readily detected, except with the aid of a microscope. Thus in 100 worms picked out with the naked eye from a suspension of intestinal contents and mucosa, not a single male was found; whereas a count made with the microscope of samples of the same material showed 31 per cent males.

Counts of 100 or more worms from the intestines of four rats of the present series showed the percentage of males to vary from 31 to 41 per cent seven or eight days after injection. In a total of 446 worms, 160, or 36 per cent, were males. An approximation of a 1:2 ratio was thus found in these animals. Rats killed seventeen or eighteen days after ingestion of infected muscle showed practically the same ratio, although only few worms were found. It is of interest to note that in one rat in which a single male was found unaccompanied by any females, numerous larvae were found in the striated muscles showing that this male had outlived one or more females which had been present.

EXPLANATION OF PLATE

Fig. 1.—A cross section of male and female *Schistosoma haematobium* situated in a distended vein at the juncture of the submucosa and muscular wall of the bladder. This vessel is evidently occluded by the inflammation which the worms' presence has excited. The intestinal ceca of the female are distended with deeply stained material to the right of which is the ovary.

Fig. 2.—Male and female *S. haematobium* in longitudinal section. In the upper portion of the sectioned worms to the right a row of eggs is visible in the tubular uterus of the female. The acetabulum of the male is apparent, directed inwardly near the anterior extremity of the worms at the left. These worms are situated in veins beneath the submucosa, in this instance 4 or 5 mm. from the surface of the mucosa. The inflammatory changes in the latter are apparently due to the presence of numerous ova, for these are found surrounded by collections of exudate with which they are evidently discharged during the contraction of the bladder. Many ova also fail to reach the surface but become imbedded in the tissue where they are eventually destroyed, the shells persisting as foreign bodies.

PLATE

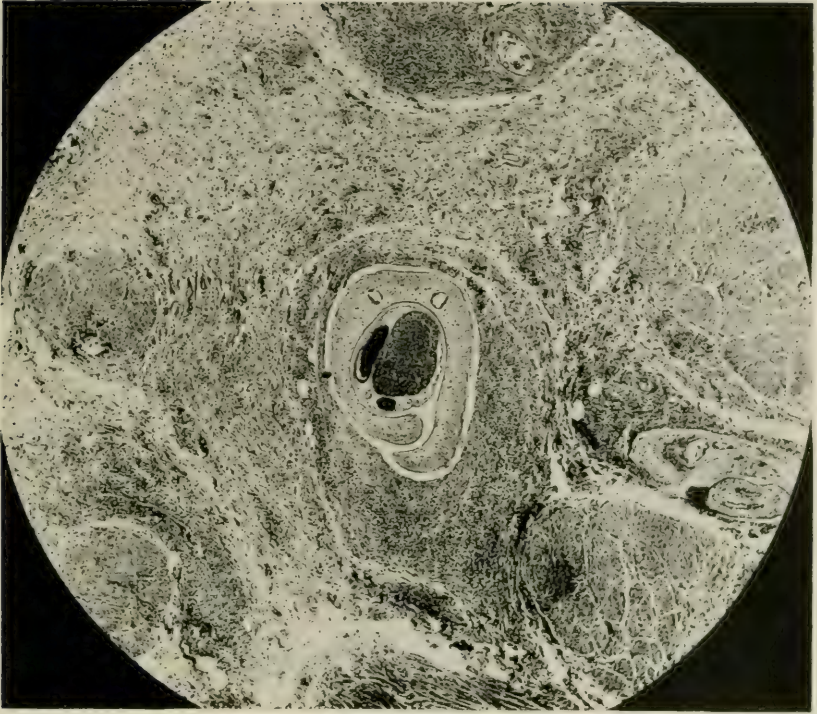


Figure 1

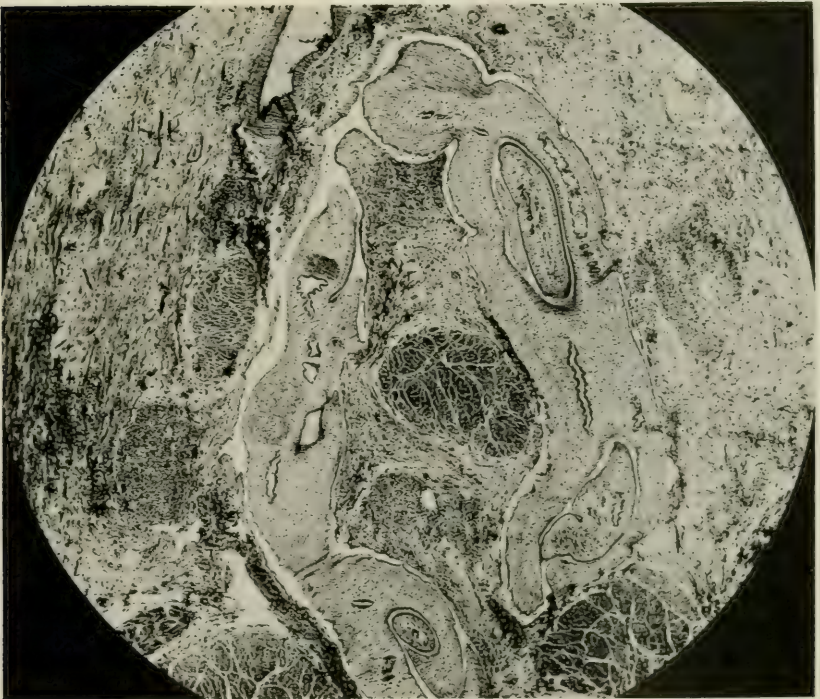


Figure 2

In mice fed with relatively small numbers of encysted larvae and killed four, five, six, seven and nine days later, there is considerable variation in the sex ratio, evidently on account of the small numbers of adult worms present in each animal. Including trichinellae subjected to radiation, together with those of the control mice, there were 516 counted, and 159, or 30.8 per cent of these were males. Considering the non-radiated separately, there were 167, of which 42, or 25 per cent, were males. There was no marked diminution in the number of males from the fourth to the seventh day, and the number counted on the ninth day is too small to be of significance. The sex ratio of one male to two females is thus also approximated for this parasite in the mouse.

Disappearance of Adult Worms from the Intestine.—Whereas the males are said to diminish in number after copulation, which is accomplished by the second or third day after ingestion, it is stated that the females may persist for five weeks or longer. Cohnheim claims to have observed trichinellae in great numbers up to the seventh week; Kratz found them seventy-seven days, and Leuckart twelve weeks after ingestion. In experimental animals the embryos are said to be liberated from adults for five to seven weeks after the ingestion of trichinous meat (Fantham, Stephens, and Theobald, 1916). It would appear from the findings in the rats of the following experiments that the adult worms disappear much earlier than the above observations by various authors would indicate. The data collected are presented in the following table.

TABLE OF RESULTS

Rat	Period of Infection	Number of Adult Trichinae	Males	Females	Remarks
Wild 4853A.....	19 days.....	None.....	Only a portion of intestine examined
Wild 4853B.....	19 days.....	None.....	Only a portion of intestine examined
White 4965 A.....	25 days.....	Present....	Present....	Present....	
White 5590.....	7 days.....	Numerous..	31.5%	68.5%	146 counted
White 5592.....	17 days.....	One.....	One.....	Entire intestine examined
White 5618.....	7 days.....	Estimated 600	36%	64%	100 counted
White 5619.....	8 days.....	Estimated 800	37%	63%	100 counted
White 5620.....	8 days.....	Estimated 1,000	41%	59%	100 counted
White 5622.....	18 days.....	None.....	Ten inches of small intestine examined
White 5623.....	18 days.....	One.....	One.....	Ten inches of small intestine examined
White 5624.....	18 days.....	Two.....	Two.....	Ten inches of small intestine examined
White 5625.....	18 days.....	None.....	Ten inches of small intestine examined
White 5626.....	18 days.....	None.....	Ten inches of small intestine examined

Numerous larvae were found in the skeletal muscles of all the negative rats showing that the adult worms had been present in the intestine of each. In one rat adult worms of both sexes were still present twenty-five days after ingestion, but in general their paucity or absence is notable in the animals killed later than the sixteenth day. In the last group, each animal of which was fed an equal amount of trichinous muscle, the most striking differences are shown with respect to the number of worms present in the rats killed seven and eight days and in those killed eighteen days later. This would indicate that this parasite's span of life in the intestine of the rat is rarely over three weeks, although there may occasionally be individuals persisting longer.

In the following experiments radium emanation was used. Since this substance is transformed at a known rate, the amount of radiant energy available is constantly diminishing (approximately one half in four days), so that the dosage is greatest at the beginning of the exposure.

1. RADIATION OF THE ABDOMEN FROM THE SURFACE OF THE BODY

In considering the effects of radiation on *Trichinella spiralis*, both the failure to develop as determined by the number present and the retardation of development as indicated by the absence of worm-shaped embryos in the females seven days after feeding were taken into account. Although various degrees of maturity were met with at this time, the presence or absence of worm-shaped embryos could be readily determined and served as a useful, although arbitrary, index. Small and evidently poorly developed males were also met with, but since they furnish no prominent feature by which their stage of development could be judged, they are not in this respect taken into consideration.

EXPERIMENT 1

February 18, 1916. Equal amounts of muscle containing encysted larvae were fed to four healthy rats and two of these served as controls, while the other two were radiated from the surface of the abdomen. Radium emanation enclosed in capillary glass tubes with 0.1 mm. of steel, 1 mm. of silver and a layer of adhesive plaster for filtration was used. This applicator was moved each day to a new area on the abdominal wall, from the entire extent of which the fur had been removed. One rat, 5591, which was radiated in this manner with a tube of 8.8 millicurie strength from the second day following ingestion of trichina, died six days later; that is, seven days after infection. An additional tube containing 6.4 mc. was added five days after feeding and two days before death. Another rat, 5593, was radiated in a similar manner with a very weak tube (3 mc.) from the sixth to the tenth day and with a 9.6 mc. tube from the tenth to the seventeenth day, when it was killed and the number and condition of intestinal trichinae determined.

TABLE OF RESULTS—EXPERIMENTS 1 AND 2

Trichina-Fed Rat	Treatment	Death	No. of Worms Found			Remarks
			Total	Males	Females	
Experiment 1						
5590	None (control).....	Killed 7 days later.....	Numerous.....	31.5%	68.5%	Females 2% without embryos
5591	Ra. 8.8 mc. from 24 day.....	Dead 7 days later.....	Numerous.....	33.7%	66.3%	Females, 32% without embryos
5592	None (control).....	Killed 17 days later.....	One.....		One.....	This female was immature
5593	Ra. 3 mc. from 6 to 10th day and 9.6 mc. from 10th day.....	Killed 17 days later.....	316	31 %	69 %	Worms large and apparently normal
Experiment 2						
5617	Ra. 11.8 mc. from 1st day.....	Dead 7 days later.....	Two.....		Two.....	Neither contains embryos
5618	None (control).....	Killed 7 days later.....	600±	36 %	64 %	Females, 1.5% without embryos
5619	None (control).....	Killed 7 days later.....	800±	37 %	63 %	
5620	None (control).....	Killed 7 days later.....	1,000±	41 %	59 %	
5621	Ra. 2 mc. from 8th day and 15.5 mc. from 11th day.....	Dead 16 days later.....	31	51.5%	48.5%	
5622	None (control).....	Killed 18 days later.....	None.....			
5623	None (control).....	Killed 18 days later.....	One.....			
5624	None (control).....	Killed 18 days later.....	Two.....		Two.....	
5625	None (control).....	Killed 18 days later.....	None.....			
5626	None (control).....	Killed 18 days later.....	None.....			

EXPERIMENT 2

March 10, 1916. Ten normal rats were fed with equal amounts of trichinous muscle mixed with bread and milk. Radiation was commenced at once with Rat 5717, a tube of 11.8 mc. strength being used. This animal was found dead at the end of seven days. Another rat was radiated with a weak tube (2 mc.) from the eighth day and also with a stronger dose (15.5 mc.) from the eleventh day. It died 16 days after infection. The results obtained in both experiments are combined in the table on the preceding page.

While these results failed to demonstrate that radiation is of therapeutic value in the treatment of trichiniasis, they indicate that it appreciably modifies the development of the parasite in the intestine. Radiation after the females have become ripe, that is after the sixth day, fails to affect an earlier disappearance of trichinellae, or to produce distinguishable injury to them. In fact, the worms appear to persist longer and to be unusually large and well developed in the late radiated animals. That larvae had continued to be liberated from the female worms was shown by the presence of very small as well as partially developed worms in the striated muscles of these rats. Although the control rats killed seventeen and eighteen days after feeding on trichinous meat furnish few or no adult trichinellae in the intestine, numerous larvae were found in the skeletal muscles of all showing that infection had occurred.

Early radiation apparently had a greater effect on the development of trichinellae in the intestine. Radiation of the rat's abdomen from the second day after the ingestion of the cysts resulted in a retardation of development as shown by the number of immature females; 32 per cent of these showed no fully formed embryos as compared with 2 per cent in the control animals. Females were observed in the radiated rat which were so backward in their development that they were considerably smaller than normal males, although seven days had elapsed since their ingestion. There appeared to have been no general failure of the immature females to become inseminated, although this may have been accomplished later than normally. In only a few small undeveloped females was the receptaculum seminis not filled with spermatozoa. Subsequent experiments have shown that under normal or ordinary conditions the worms almost without exception are fully developed seven days, and usually six days, after they have entered the alimentary tract. Only a few larvae were found on careful search in the diaphragm of the rat radiated from the second day, whereas they were present in great numbers in the diaphragm of the control rat. Still earlier radiation, that is, from the time of the ingestion of the encysted larvae, appears to be even more effective, and in the animal in which this was carried out only two females, neither of which con-

tained embryos, were found. The three rats which served as controls, for this animal each showed numerous well developed worms estimated at 600, 800, and 1,000, respectively.

Since the radiation employed was fatal to three of the four trichinous rats, the possibility that the injury to the host might indirectly affect the life of the parasites may be considered. That changes in the host resulting from radiation do not tend to destroy the worms is shown in the late radiated rat in which adult worms persisted longer than in the controls.

II. THE RADIATION OF ENCYSTED LARVAE BEFORE INGESTION

It appeared important, in order to estimate the dosage appropriate for the employment of shorter radium rays, to radiate the larvae before they were fed to the animals. For this purpose the filtration through the millimeter of silver was dispensed with and capillary glass tubes of emanation enclosed in steel tubes having walls 0.1 mm. in thickness were employed. Under-estimation of the effects of the short rays necessitated repeating the experiment several times. In Experiment 3, which is not presented in tabular form, none of the larvae in meat radiated with a 5.9 mc. tube for six and for three days developed when fed to mice. Control mice fed with untreated meat in every case showed trichinellae when killed later on.

For the purpose of making the observations more accurate, equal numbers of encysted larvae were fed to each animal in all subsequent experiments. The encysted larvae were radiated by wrapping strips of mouse diaphragm around the steel tube containing the emanation, in this way ensuring fairly uniform radiation of all portions of the muscle. The layer of muscle around the steel tube nowhere exceeded 1.5 mm. in thickness. The strips of diaphragm were subdivided when necessary so that an equal number of cysts could be fed to each mouse. This was accomplished by placing the bits of diaphragm in the mouth of the animal and holding the latter until the material was swallowed.

EXPERIMENT 4

April 14, 1916. Twelve mice were employed in this experiment. Two of these served as control animals, being fed each with 40 trichina cysts. The other ten were each fed 40 cysts which had been radiated for different periods with 5.5 mc. of emanation enclosed in a capillary glass tube and filtered through 0.1 mm. of steel. Tissue radiated was nowhere more than 1.5 mm. in thickness. Six of the animals were killed four and five days after this feeding, and a count made of the number of *Tr. spiralis* present in the small and large intestine of each. The other six were allowed to live for a longer period and the muscles were then examined to determine whether infection had taken place.

TABLE OF RESULTS—EXPERIMENT 4

No. Mouse	Larvae Radiated with 5.5 Mc.	Each Fed 40 Larvae	Killed	Mature		Immature		To- tal
				♂	♀	♂	♀	
5699	Control untreated.....	April 14.....	April 20.....	3	10	0	1	14
5700	Control untreated.....	April 14.....	April 21.....	0	10	0	0	10
5691	3 hrs. at 33 C.....	April 14.....	April 20.....	0	0	0	0	0
5692	3 hrs. at 33 C.....	April 14.....	April 20.....	0	0	0	0	0
5693	6 hrs. at 33 C.....	April 14.....	April 20.....	0	0	0	0	0
5694	6 hrs. at 33 C.....	April 14.....	April 21.....	0	0	0	0	0
5695	12 hrs. at 33 C.....	April 14.....	May 4.....	Muscles negative				0
5696	12 hrs. at 33 C.....	April 14.....	May 4.....	Muscles negative				0
5697	24 hrs. at 33 C.....	April 14.....	May 4.....	Muscles negative				0
5698	24 hrs. at 33 C.....	April 14.....	May 4.....	Muscles negative				0
5701	48 hrs. at 33 C.....	April 14.....	May 4.....	Muscles negative				0
5702	48 hrs. at 33 C.....	April 14.....	May 4.....	Muscles negative				0

EXPERIMENT 5

April 21, 1916. Fourteen mice were employed, two receiving untreated cysts, the others receiving equal numbers of cysts which had been radiated for different periods of time at room temperature. The technic employed was the same as that in the preceding experiment but shorter exposures were made. The radiated tissue was nowhere more than 1.2 mm. in thickness. The mice were all killed five or six days after the feeding.

TABLE OF RESULTS—EXPERIMENT 5

No. Mouse	Larvae Radiated with 7.1 Mc.	Each Fed 40 Larvae	Killed	Mature		Immature		To- tal
				♂	♀	♂	♀	
5738	Control untreated.....	April 21.....	April 26.....	0	3	0	1	4
5739	Control untreated.....	April 21.....	April 27.....	2	8	0	0	10
5726	2½ minutes.....	April 21.....	April 26.....	1	1	2	8	12
5727	2½ minutes.....	April 21.....	April 27.....	6	10	0	0	16
5728	5 minutes.....	April 21.....	April 26.....	3	5	3	2	13
5729	5 minutes.....	April 21.....	April 27.....	1	3	0	6	10
5730	10 minutes.....	April 21.....	April 26.....	1	8	2	4	15
5731	10 minutes.....	April 21.....	April 27.....	0	0	0	0	0
5732	20 minutes.....	April 21.....	April 26.....	0	0	0	0	0
5733	20 minutes.....	April 21.....	April 27.....	7	12	0	0	19
5734	40 minutes.....	April 21.....	April 26.....	0	0	0	0	0
5735	40 minutes.....	April 21.....	April 27.....	1	1	0	0	2
5736	80 minutes.....	April 21.....	April 26.....	0	0	0	0	0
5737	80 minutes.....	April 21.....	April 27.....	0	0	0	0	0

It is apparent from the above experiment that radiation for eighty minutes with 7.1 mc. is fatal to the encysted larvae, but the results obtained for the next shorter periods are rather variable, one positive and one negative result being obtained in each of the three successive periods. Radiation for two and one-half and for five minutes appears not to have been markedly injurious. It was thought possible that since certain portions in the length of the steel tube employed were less

radioactive than others, certain portions of the diaphragm exposed may have been subjected to less radiation accounting for the irregularity of the results obtained. On account of this it was considered necessary to repeat this experiment, paying especial attention to the equal radiation of all parts of the material used.

EXPERIMENT 6

May 1, 1916. Ten mice were employed, two served as controls, and the others received equal numbers of encysted larvae radiated for various periods. These were killed four and five days after this feeding and the intestine examined for *Tr. spiralis*.

TABLE OF RESULTS—EXPERIMENT 6

No. Mouse	Larvae Radiated with 6.6 Mc.	Each Fed 40 Larvae	Killed	Mature		Immature		To- tal
				♂	♀	♂	♀	
5749	Control untreated.....	May 1.....	May 5.....	4	0	0	1	5
5750	Control untreated.....	May 1.....	May 6.....	1	3	0	8	12
5747	20 minutes.....	May 1.....	May 5.....	1	0	2	5	8
5748	20 minutes.....	May 1.....	May 6.....	0	0	0	1	1
5745	30 minutes.....	May 1.....	May 5.....	0	0	0	0	0
5746	30 minutes.....	May 1.....	May 6.....	0	0	0	0	0
5743	40 minutes.....	May 1.....	May 5.....	0	0	0	0	0
5744	40 minutes.....	May 1.....	May 6.....	0	0	0	0	0
5741	60 minutes.....	May 1.....	May 5.....	0	0	0	0	0
5742	60 minutes.....	May 1.....	May 6.....	0	0	0	0	0

From the three preceding experiments the lethal dosage of radiation for encysted trichinae is determined. They are made non-infectious for mice by radiation with 6.6 mc. filtered through thin glass and 0.1 mm. of steel in an exposure of thirty minutes. The cysts exposed were at a distance of not over 1.5 mm. from the source of radiation. It would be of interest to learn more concerning the effects of this amount of radiation on encysted larvae whether they are killed outright, or the cyst made more resistant to the digestive juices or the larvae injured to such an extent that they are passed from the alimentary tract before they can recover sufficiently to maintain their existence. In all of the present experiments only the immediate result of radiation as shown by the absence or by the arrested development of worms, has been determined. It would be of considerable interest to note whether any remote or late changes are brought about, but this probably would be more readily determined in a free-living rather than in a parasitic species.

III. THE DIRECT RADIATION OF TRICHINELLA SPIRALIS FROM THE INTERIOR OF THE ALIMENTARY TRACT

Since the short rays were found to be effective in the destruction of the larvae the direct radiation of the worms from the interior of the intestine was next undertaken. Through radiating the interior of

the intestine by means of tubes of emanation fed to the animal it was hoped to utilize very short rays. The movement of the intestinal contents was expected to prevent undue burning of the mucosa of the small intestine and the incorporation of the tube in more or less solid fecal material was hoped to protect the wall of the large intestine from serious injury. Minute tubes of emanation were prepared by Doctor Duane. Since these measured only from 2 to 3 mm. in length and a fraction of a millimeter in diameter, they could be readily introduced into the stomach of the mouse. In order to accomplish this, a large syringe needle, the point of which had been ground off square, covered with paraffin and dipped in oil so that it could be readily passed down the esophagus of the mouse, was used. The emanation tube having been placed in the needle, it was forced by a plunger into the stomach of the mouse. It could readily be determined at any time whether the tube had been passed from the intestine or was still in the body of the mouse by placing the latter on the ionization chamber of the measuring apparatus.

EXPERIMENT 7

June 27, 1916. Nine mice were each fed 40 trichina cysts in bits of mouse diaphragm. Minute glass tubes of emanation were introduced into the stomach of three of these, one receiving a tube on the first day, another on the second day, and another on the third day.

TABLE OF RESULTS—EXPERIMENT 7

No.	No. Fed	Radium Fed	Died	No. Tr. Spiralis
5803	80 cysts.....	3.1 mc. 7 hours later.....	Killed 5 days....	24 (4 immature)
5804	80 cysts.....	4.1 mc. 28 hours later.....	Dead 6 days....	57
5805	80 cysts.....	3.2 mc. 51.5 hours later.....	Dead 6 days....	46
5806	80 cysts.....	Control.....	Killed 5 days....	33
5807	80 cysts.....	Control.....	Not examined	
5808	80 cysts.....	Control.....	Not examined	
5809	80 cysts.....	Control.....	Not examined	
5810	80 cysts.....	Control.....	Not examined	
5811	80 cysts.....	Control.....	Not examined	

In the first mouse (5803) the tube failed to pass from the intestine during the four days and fourteen hours which elapsed before it was killed on account of its weak appearance. The tube fed the second mouse (5804) was still in its body after three days, but had been passed when found dead two days later. The tube fed the third animal (5805) was passed within forty-eight hours. Notwithstanding the small dosage, 3.2 mc., for so short a period, this animal died before the end of four days after its introduction. This mouse presented a reddened area in the wall of the large intestine. The others showed no local effects of the radiation, but all showed a striking shrinkage in the size of the spleen so characteristic of radiated animals. These results show unmistakably that in the mouse even fatal doses of radium emanation acting

from the interior of the intestine fail to prevent the development of *Tr. spiralis*. It is quite apparent that this parasite is not especially vulnerable to rays which have an immediate destructive influence on the lymphoid tissue of the host.

The results of the foregoing experiments are thus rather discouraging with respect to the application of radiation to parasitic worms. It is rather remarkable that *Tr. spiralis* is so little affected after commencing its development within the body when it, in its encysted state, is so quickly destroyed by radiation outside the body. Whether internal radiation would accomplish more in larger, more resistant animals remains to be determined. The acceleration of the passage of radium emanation through the large intestine by the employment of a cathartic might be of value, but was not tried in these experiments. It would appear, however, that, under the conditions of the above experiment, radiation destroys the resistance of the animals before the parasite is markedly affected.

These results do not, therefore, furnish an experimental basis for the treatment of schistosomiasis by radiation from the interior of the bladder. Although this treatment would for some reasons appear especially applicable to this disease, it would probably be impossible, with the short rays, to reach all the adult worms, for they are frequently situated at a considerable distance from the surface of the mucosa. Treatment of this disease by radiation, although justified on theoretical grounds, must for the present be regarded as experimental in character, and, even if no untoward results occur, it will be difficult to determine just what has been accomplished. On the other hand, the localization of the worms and the relatively large size of the host are distinctly in favor of this form of treatment. The fact that it is a disease frequently attended with serious complications would appear to warrant cautious treatment by radiation, provided that the experimental nature of the treatment is explained to the patient, and provided that changes in the local condition may be followed by cystoscopic examination and by observations with reference to the number of ova discharged in the urine.

SUMMARY

By radium radiation from the surface of the abdomen of the rat the injury of fully developed *Trichinella spiralis* has not been accomplished. These worms appeared well developed and persisted longer than in controls.

Similar treatment from the second day after the ingestion of cysts has apparently resulted in a retardation of development, 30 per cent of the females being immature.

In a rat radiated in this manner from the time it was fed trichinous meat, only two immature worms were found seven days later, indicating that radiation of the larvae before they have entered upon their period of development free in the intestine is fatal to them.

The radiation of encysted larvae with 6.6 mc. of emanation, 0.1 mm. steel filtration, at a distance of not over 1.5 mm. for thirty minutes renders them non-infectious for mice.

Radiation from the interior of the intestine—employing emanation in minute glass tubes for the utilization of short rays—in amounts sufficient to cause the death of the mice employed has not prevented the development of *Tr. spiralis*.

These results fail to furnish an experimental basis for the treatment of schistosomiasis by radiation, so that if the latter is attempted it should be regarded as an experiment rather than an approved mode of procedure.

Observations on the life history of *Trichinella spiralis* made in the course of these experiments indicate that certain points emphasized in books of reference do not apply to the development of this parasite in rats and mice.

Trichinella spiralis is found only in small numbers in the duodenum and jejunum of rats and mice which show great numbers in the lower portion of the small intestine. It is also occasionally found in the cecum and large intestine.

The life of this parasite is comparatively short in the rat, and it is found to have disappeared or is present only in small numbers eighteen days after infection.

No evidence has been obtained that the males disappear early in the infection. A sex ratio of 1 ♂ : 2 ♀ observed six and seven days after infection has shown no marked change for the ten days following. A male *Trichinella* has been found in a rat from which all females had disappeared.

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NOTES ON SOME NEMATODES FROM FRESH-WATER FISHES *

HENRY B. WARD AND THOMAS B. MAGATH

The parasitic nematodes are of conspicuous importance in the field of human disease and also in diseases of the domestic animals, and in his treatise on fish diseases, Hofer (1906), discussing the significance in fish culture of parasites and parasitic diseases, states that among them the nematodes outrank all others in number of types. Yet as fish parasites these forms are almost unknown in North America, and references to them are confined to a few brief notes, almost all of which came from the pen of the distinguished Philadelphia microscopist, anatomist, and parasitologist, Joseph Leidy, whose pioneer work published between 1850 and 1886 includes many records of great value on this group.

In this little-explored field the senior author has been making observations for many years and in collaboration with the junior author was led recently to undertake an extended study of nematode parasites from North American fresh-water fishes which has yielded a number of new and interesting forms; these are briefly described here in advance of the appearance of the complete article in which will be given fuller data on the structure and relationships of these species. Especial thanks are due the United States Bureau of Fisheries for aid in securing material.

It is interesting to note that among the eight forms described as new species, three fall within new genera and five agree sufficiently with European forms to be listed in already existing genera. Seven out of the nine forms described in this paper come within the limits of the Spiruroidea, so that this superfamily appears to hold a prominent place among parasites of fresh-water fishes.

Cystidicola Fischer von Waldheim 1797. — The type species *C. farionis* from the air-bladder of the trout and other fishes is a common form in Europe; it has also been reported from the lake trout of Lake Erie. Leidy (1886) described a parasite of the air-bladder of the lake trout as *Filaria stigmatura*. We have the same parasite from the white fish, lake trout, and lake herring in Lake Erie, Lake St. Clair and Lake Michigan. It is clearly not a *Filaria*, but belongs to the genus *Cystidicola*, and should be called *C. stigmatura*. Two small

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uncinate lips, a short buccal capsule or tubular pharynx followed by a long esophagus divided into an anterior muscular and a posterior glandular region, indicate the position of this form in the superfamily Spiruroidea created by Railliet and Henry (1916) and in the family Camallanidae established by the same authors.

Camallanus Railliet and Henry 1915.—Among the commonest and best known parasites of fresh-water fish in Europe is the so-called Hooded Worm, easily recognized by its horny oral armature in the form of heavy ribbed valve-shaped lips of dark brown material with corner anchors of trident form. More than fifty names have been given these worms which most often have been assigned to the genera *Dacnitis* and *Cucullanus*, but have recently been definitely located in a new genus, *Camallanus*, by Railliet and Henry (1915a). Though in habit hookworms by virtue of their persistent attachment to the intestinal wall, they do not possess a buccal capsule like all true hookworms; the valve-shaped lips form powerful lateral jaws that grasp the tissue, whereas the true hookworms possess a hollow cup into which the tissue is drawn by suction. Two species have been found in North American fresh-water fishes. The first is

Camallanus ancylodirus nov. spec.—The head (Fig. 4) is bent sharply ventrad, whence the specific name. The mature female is 25 mm. long by 0.56 mm. in maximum diameter. The caudal tip is bluntly conical, and 0.45 mm. in front of it lies the anus. The vulva is three-fifths of the length from the anterior end. The trident in the oral armature has three or rarely four prongs, irregular in form and 0.21 mm. long. The lips measure 0.142 to 0.168 mm. long by 0.18 to 0.187 mm. broad. The uterus, loaded with minute embryos, fills the entire body save at the extreme ends. The anterior region of the esophagus is 1.416 mm. long and 0.096 to 0.15 mm. broad; the posterior region measures 1.368 by 0.072 mm.

Mature males measure 15 mm. in length by 0.38 mm. in breadth at the center of the body. The lips are 0.126 mm. long by 0.12 mm. wide, and the trident arms 0.18 mm. long. The anus (Fig. 10) lies 0.156 mm. from the extreme tip. The caudal alae are 0.92 mm. long. The two spicules (Fig. 11) are nearly equal in length, but one is only half as heavy as the other. The posterior end is rolled in a half circle and the number of the caudal papillae could not be determined.

This parasite came from the intestine of the German carp at Fairport, Iowa, and is the first reported from that host on this continent. Evidently it is a native species that has accommodated itself to this new host, and its original host is as yet unknown. Instances are infrequent in which a host is known to have acquired an entirely new parasite.

Camallanus orycephalus nov. spec.—Only females were found. Length up to 25 mm. and maximum breadth 0.27 mm. The anterior end (Fig. 3) is perfectly straight and much smaller than the preceding species. The body tapers regularly from the center about equally in both directions. A small but distinct constriction, or "neck," lies just behind the oral tridents and conforms to the curvature of the trident branches. The muscular esophagus is 0.47 mm. long and 0.085 mm. in diameter at the narrowest point, expanding near the end to 0.105 mm.; the second region of the esophagus is 0.57 mm. long, of nearly equal diameter throughout and as broad as the maximum breadth of the muscular esophagus. The vulva is located at the anterior margin of the middle third of the body. This species was taken from the intestine of the white bass and of the black crappie at Fairport, Iowa.

Cucullanus O. F. Müller 1777.—As Railliet and Henry have shown recently (1915a), the forms which rightly belong here are those to which the name *Dacnitis* Duj. 1845 has often been applied. They are characterized as follows:

Anterior end bent dorsad. Mouth elliptical with major axis dorso-ventral, bounded by two lateral valves recalling those of *Camallanus*. Esophagus pestle-shaped without bulb. Males without caudal alae; two equal spicules and an accessory piece. Preanal sucker without chitinous ring. Female with vulva near center. In alimentary canal of fish.

The genus was placed in the Heterakidae by Railliet and Henry chiefly because of the preanal sucker of the male. However, a ventral sucker is by no means confined to this family, and this one has no marginal ring such as is present in other Heterakidae; finally, the three lips of the Heterakidae are wanting. Accordingly, we have placed the genus in the Spiruroidea with which it agrees in the lateral valves at the mouth and the double esophagus, both characteristic of that group. The genus is the sole known representative of a family, the Cucullanidae, which differs from all other forms in the Spiruroidea through the possession of the ventral sucker in the male.

Cucullanus clitellarius nov. spec.—Body generally uniform in diameter, except for clitellar-like swelling 1.5 mm. from anterior tip (Fig. 5). Head bent dorsad 60 to 90 degrees. On each oral margin three papillae. Oral valves 0.45 by 0.32 mm. in female, and 0.33 by 0.24 mm. in male.

Males 10 to 11 mm. long by 0.38 mm. broad. Esophagus 1.45 mm. long and 0.12 to 0.22 mm. wide. Caudal region (Fig. 9) bent in a single turn. Ventral sucker 0.51 mm. anterior to anus, 0.1 mm. in diameter. From anus to tip of body 0.39 mm. Spicules 1.62 mm. long,

0.035 mm. broad, shaped like a gouge; accessory piece dagger-shaped, 0.06 long by 0.015 mm. broad. Two small papillae just in front of anus; four pairs of postanal papillae, of which two pairs are large, rounded, and only 0.012 mm. from extreme tip.

Females 12 to 17 mm. long, 0.5 mm. broad. Esophagus 1.6 mm. long and 0.13 to 0.32 mm. wide. Distance from anterior end to vulva from five-ninths to two-thirds total length. Uterus and ovary double. Ova 63 by 46 μ .

Parasitic in intestine of lake sturgeon (*Acipenser rubicundus*) in Lake St. Clair.

This genus has been heretofore the sole representative of its family, but among parasites in fresh-water fishes we have met another type that is sufficiently related to fall within the limits of the family, and yet cannot be brought within the limits of the same genus. To receive it a new genus has accordingly been created with the following characteristics:

Dacnitoides nov. gen.—Much like *Cucullanus*, except that head is not flexed and body is straight; spicules lack accessory piece, and only a single ovary is developed. A well developed intestinal cecum is present.

Dacnitoides cotylophora nov. spec.—Males 4 to 6 mm. long and 0.2 mm. broad. Mouth dorso-ventral, bounded by lateral valves. Cuticular ridge with three papillae at extreme anterior margin of each valve. Esophagus 0.5 to 0.6 mm. long, 0.06 to 0.12 mm. broad, distinctly divided into two regions; anterior region 0.2 mm. long. Intestine large, provided with dorsal cecum extending anteriorly to junction between two regions of esophagus. Ventral sucker (Fig. 7) 0.41 mm. in front of anus, which is 0.12 mm. from posterior tip. Spicules 0.89 mm. long and only 5 μ broad. Caudal papillae: one pair on anterior margin of sucker, four pairs between sucker and anus, four pairs of postanal papillae and one single median papilla immediately in front of anus.

Females 4 to 5.5 mm. long by 0.28 mm. in width at vulva; body distinctly short and heavy. Anterior end (Fig. 6) rounded, posterior end acutely pointed. Anus 0.14 mm. from posterior tip, with four slender spines halfway between. Vulva about five-eighths of total length from anterior end. Anterior and posterior uterine branches, but latter terminates blindly, and only former has an ovarian tube at end. Eggs measure 65 by 40 μ , and contain embryos in early cleavage stages.

This parasite was common at Lake St. Clair in the intestine of the yellow perch (*Perca flavescens*), and more rarely of the wall-eye (*Stizostedion vitreum*).

In that this species possesses an intestinal cecum it resembles the family of the Heterocheilidae which Railliet and Henry established

among the Ascaroidea on the basis of the development of such an organ. In other anatomical features it departs as widely from that family as it does from the Heterakidae.

An interesting parasite was taken from the intestine of the bowfin, or fresh-water dog-fish, *Amia calva*, both in Lake St. Clair, Michigan, and at Fairport, Iowa. It is of a very generalized character and hence difficult to define except in negative characters, or to locate in the system. While we are inclined to place it in the Spiruroidea under the family Spiruridae, we must confess that this decision is open to criticism, and it may have to be made the representative of a new family standing half way between the Ascaroidea and the Spiruroidea. It must certainly be made a new genus characterized as follows.

Haplonema nov. gen.—Body rather robust, but not large; anterior end bent or coiled, without lips or papillae, but with lateral alae ("wings"). No buccal capsule; esophagus muscular, without bulb, divided into two regions by partition near center. Posterior end of female straight, or slightly curved behind anus, with two minute papillae. Posterior end of male without bursa or alae, with two equal spicules of moderate length. Two pairs of preanal papillae and three pairs of postanals. Ovary laid in transverse loops ventral to intestine in both anterior and posterior regions. Uterus with anterior and posterior branches, vulva near center of body. Oviparous.

Haplonema immutatum nov. spec.—Males less frequent than females, measure about 9.5 mm. in length by 0.2 mm. in breadth anteriorly and 0.18 mm. posteriorly. Females about 15 mm. long by 0.31 mm. in maximum breadth in the anterior region. Vulva five-eighths of length from anterior tip.

Anterior end (Fig. 1) bent in a half circle with lateral cuticular folds extending back 2.5 or 3 mm. from anterior tip. Esophagus prominent, muscular, 0.65 mm. long in male and 0.8 mm. in female; width of esophagus, 0.06 mm. anteriorly and 0.1 mm. near its posterior end. Esophageal partition inconspicuous, near center of length; two regions alike in structure.

Spicules (Fig. 2) two, equal, flat, ribbon-like, 0.75 mm. long, by 0.02 mm. wide. Eggs abundant, with moderately thick, smooth shells; average size 65 by 45 μ .

The genus *Spinitectus* was established by Fourment in 1883 to contain parasites of fishes characterized by circles of retrorse spines on the posterior margins of transverse rings. To the four species known in Europe we add a new form common in some places here.

Spinitectus gracilis nov. spec.—Mature females 11 to 19 mm. long; body divided more or less distinctly into slender transparent anterior region about 6 mm. long and 0.066 mm. wide, and larger, darker, more

opaque posterior region about 12 mm. long and 0.14 mm. broad, crowded with internal organs, especially the uterus gorged with eggs. Tail abruptly conical, 0.096 mm. from anus to tip in female, only 0.066 in male. Vulva three-fourths length of body from anterior end, inconspicuous.

Spinous rings begin 0.12 mm. from anterior tip. First 6 to 8 rows more prominent than those later. Subsequent rows (up to 28 or 30) smaller, closer, with spines much lighter and shorter; last rows difficult to detect. Total about 130 rows. Rings about 0.03 mm. apart at anterior end, and contain 40 to 50 spines in each circle. Largest spines 8μ long, smallest less than half as long.

Male 12 mm. long by 0.042 mm. in diameter in anterior region and 0.75 mm. at widest point. Posterior end coiled in spiral with 2 to 3 turns. Narrow lateral alae 0.33 mm. long, near caudal tip, not supported by papillae or ribs. On ventral surface, 1 mm. anterior to anus a series of 4 to 8 longitudinal rows of small ridges, each about 5μ long and 3μ high. Spicules very heavy, longer scoop-shaped; shorter, arcuate, oblique, probably not protrusible.

Pharynx tubular or funnel-shaped, short. Muscular esophagus narrow, 0.33 mm. long in female and 0.25 mm. in male, no marked boundary between it and glandular region. Egg-filled uterus large. Ova 41 by 24μ , with thick transparent wall and without polar processes.

Some specimens 4 to 5 mm. long by 0.35 mm. broad have spines extending even to the anal region, 175 rows in all. From the vulva a broad vagina projects antieriad bearing at its inner end anterior and posterior uterine branches.

This parasite occurs in the alimentary canal of the black crappie, sheepshead, and white bass at Fairport, Iowa. In life it is transparent, and the spinous rings are very distinct. The worms are not attached to the wall, but lie free in the lumen of the gut. The spines are encased in masses of food particles.

Railliet and Henry include in this genus as *Spinitectus cristatus* a form described by Linton from the hake as *Filaria serrata*. They use his description of the male for the genus since Fourment found no males in his material. Our form differs from Linton's in the absence here of papillae he described and in the presence here of caudal alae he neither mentions nor figures.

A nematode parasitic in the perch in Lake St. Clair is assigned without hesitation to the genus *Ichthyonema*. The genus is closely related to *Dracunculus medinensis* of man. In Europe various species are abundant, and widely distributed, but one has never been reported before this in North America. Possibly some of the worms listed as "Filaria" from American fishes really belong here. The species we have does not agree with any known form and is designated as

Ichthyonema cylindraceum nov. spec. — Male unknown, probably minute. Mature female 100 mm. long, of nearly equal diameter (0.48 mm.) everywhere. No lips or papillae. Esophagus 1.09 mm. long, 0.066 mm. in diameter. Vulva and vagina atrophied, no vestiges discernible. Uterus crowded with undeveloped ova (i. e., female unimpregnated), ova almost spherical, measure 0.044 mm. in diameter.

In abdominal cavity of *Perca flavescens*, Lake St. Clair.

The worm was delicate, semitransparent, and very fragile owing to the thin body wall. The lateral lines are broad, light colored, and conspicuous. In Europe almost half of the females found are like our material, unimpregnated owing apparently to scarcity of males. This species is of great interest from its relationship to the Guinea Worm of man.

Among the Ascaridae, Railliet and Henry (1915) have grouped those forms having either an intestinal or an esophageal cecum into a single family, the Heterocheilidae. One form we have studied falls within this group, but cannot be placed in any of the genera found in it, hence a new genus is created to contain it characterized as follows:

Hysterothylacium nov. gen.—Body without anterior tunic, but with narrow lateral alae ("wings"). Lips three, not prominent. Esophagus long, slender, with terminal spherical bulb. Intestine with short simple cecum, arising from anterior end of intestine, directed posteriad. Males with two equal curved spicules, papillae(?). Females unknown.

Hysterothylacium brachyurum nov. spec.—Length of male 32 mm., maximum width just behind center of body, 0.66 mm. Lateral fin (Fig. 8) from base of lip to esophageal bulb or further; width one quarter the diameter of body.

Esophagus 3.1 mm. long, 0.1 to 0.13 mm. wide; bulb with three teeth, cecum 0.94 mm. long, 0.08 mm. wide. Spicules equal, 0.72 mm. long by 0.045 mm. wide. Pyriform sperm-vesicle prominent. In stomach of black bass, Lake St. Clair, Michigan.

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EXPLANATION OF PLATE

Fig. 1.—*Haplonema immutatum*; head of female showing lateral fin fold, excretory pore, two regions of esophagus, esophageal valve, and intestine. $\times 72$.

Fig. 2.—*Haplonema immutatum*; tail of male showing spicules and papillae. $\times 72$.

Fig. 3.—*Camallanus oxycephalus*; head of female showing valves, trident, ring, and anterior region of esophagus. $\times 72$.

Fig. 4.—*Camallanus ancyloDIRUS*; head of female showing oral armature and two regions of esophagus. $\times 22$.

Fig. 5.—*Cucullanus clitellarius*; head of female showing valves, double esophagus, esophageal valve and intestine; also half of clitellar-like swelling. $\times 20$.

Fig. 6.—*Dacnitoides cotylophora*; head of female, showing oral armature, esophageal regions, intestine, cecum, and anterior coils of ovary. $\times 70$.

Fig. 7.—*Dacnitoides cotylophora*; tail of male, showing sucker, spicules and papillae. $\times 70$.

Fig. 8.—*Hysterothylacium brachyurum*; head of male showing lips, lateral fin-fold, esophagus, esophageal bulb, intestine, and cecum. $\times 22$.

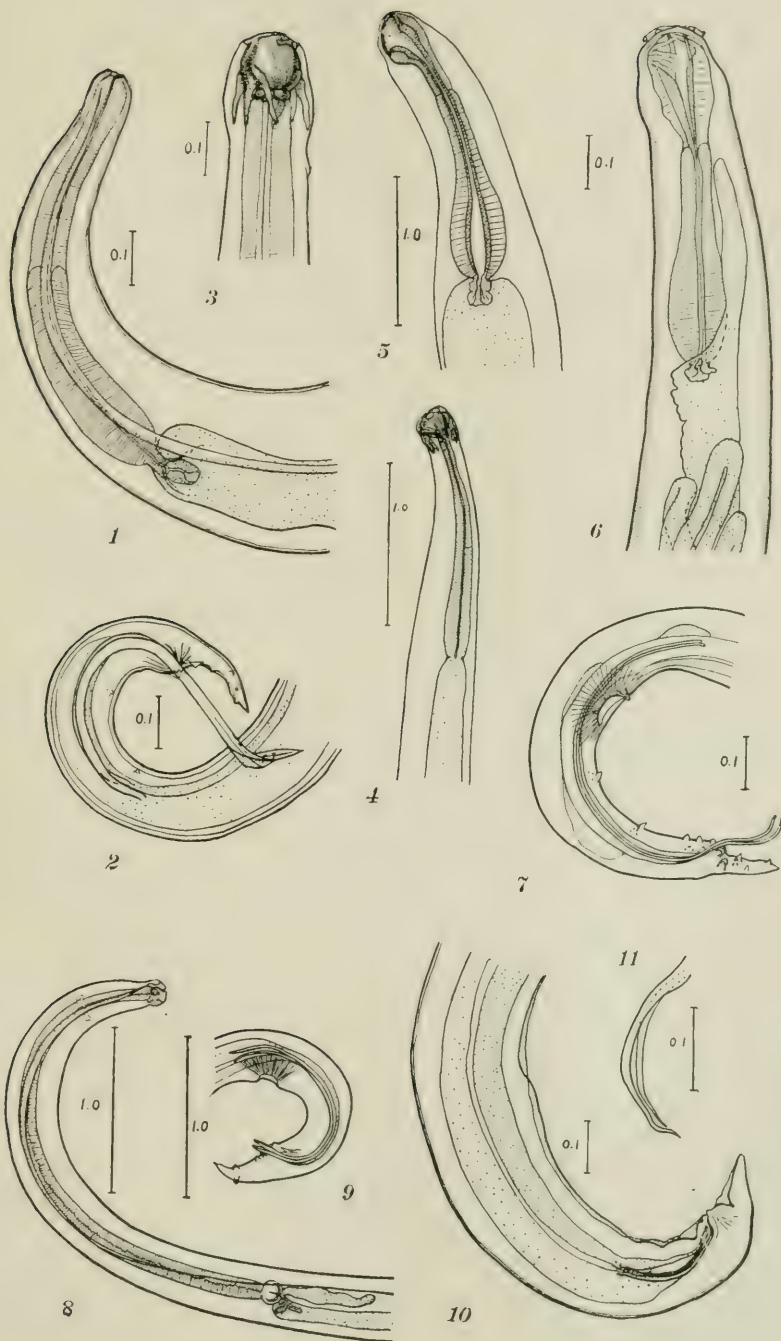
Fig. 9.—*Cucullanus clitellarius*; tail of male, showing sucker, spicules and papillae. $\times 22$.

Fig. 10.—*Camallanus ancyloDIRUS*, tail of male showing spicules and papillae. $\times 70$.

Fig. 11.—*Camallanus ancyloDIRUS*, spicules of male. $\times 110$.

All reference lines in millimeters or tenths as indicated on plate.

PLATE



OBSERVATIONS ON POLYCYSTID GREGARINES
FROM ARTHROPODA *

MINNIE E. WATSON

The following pages contain observations on already known and new species obtained by the writer chiefly during the past summer.

BULBOCEPHALUS WARDI nov. gen., nov. spec.

[Figures 1, 2, and 3]

Host: Clerid larva (Det. E. P. Felt)

Habitat: Intestine

Location: Oyster Bay, Long Island, June, 1916

Fifty or more specimens of this gregarine, mostly free trophozoites, were taken from a single Clerid larva. The sporonts are solitary and rather small, and the species is characterized chiefly by the distinct bulb in the mid-region of the large epimerite of the trophozoite.

The longest sporont seen measured 190μ in length and 45μ in width. One trophozoite exceeded in length the longest sporont, being 290μ in length. The protomerite of the sporont is slightly longer than wide, broadly rounded in front, and slightly constricted at the septum. The deutomerite is widest at the "shoulder" and tapers from thence, ending in a long blunt-pointed extremity. The average ratio of length of protomerite to total length is about 1:5; the protomerite and deutomerite are of approximately the same width.

The epimerite is unique. It consists of three parts: *a*, a broad base fitting on the apex of the protomerite, tapering at the top to form a neck; *b*, a spherical bulb, in the middle; *c*, a stout style at the apex. In other words, the epimerite consists of a stout broad-based style, bulbous in the middle. In length, it measures one-fifth to one-third the total length of the trophozoite. Complete dimensions of several epimerites are given in the table at the end of this species.

The protoplasm is dense, rendering it dark in color in both protomerite and deutomerite. The ellipsoidal nucleus is not visible in the live sporont; when stained, it is seen to be small in the sporont (larger in comparison in the trophozoite), and lies diagonally across the largest part of the deutomerite. One karyosome is present.

The writer is unable to classify this species in any known genus. The epimerite is most nearly related to that of the genus *Stylocephalus*; in this genus it consists of a more or less long, slender neck terminating

* Contributions from the Zoological Laboratory of the University of Illinois under the Direction of Henry B. Ward, No. 79.

in a large or small papilla. There may or may not be a bulbous base. The nucleus is ellipsoidal. The type species of the genus is *Stylocephalus oblongatus* (Hammerschmidt) Watson, see Watson 1916: 159.

The present specimens differ from the type species of the aforesaid genus in but one respect: The bulbous papilla of the epimerite is placed not at the apex of the cylindrical neck, but at its mid-point. In no species of the named genus is there a papilla at other than the apex. It therefore devolves upon the writer to describe a new genus in order to place the specimens found. I therefore designate it *Bulbocephalus*, and place it as follows:

Family Stylocephalidae Ellis. Sporonts solitary, epimerites varied. Nucleus ovoidal. Dehiscence by pseudocyst. Spores irregularly shaped, brown or black, in chains (Watson 1916: 47).

Type Genus *Stylocephalus* Ellis. Epimerite a dilated papilla at the end of a long slender neck. Cyst covered with small papillae and indentations. Spores hat-shaped.

New Genus *Bulbocephalus*. Epimerite a dilated papilla placed in the middle of a rather long slender neck. Cyst and spores unknown.

The species is named for Professor Henry B. Ward of the University of Illinois.

A table of typical measurements, in microns, is given herewith:

Length epimerite	60	x	x	40	30
Length protomerite (without epimerite) .	50	30	40	15	20
Length deutomerite	180	62	30	55	40
Total length sporont.....	290	192	170	110	90
Width epimerite	16	x	x	16	10
Width protomerite	42	32	40	20	18
Width deutomerite	37	45	40	28	31
Ratio length prot. (without epim.):					
total length	1:5.8	1:6.4	1:4.2	1:7	1:4.5
Ratio width prot.: width deut.....	1.1:1	1:1.4	1:1	1:1.4	1:1.7
Diameter nucleus			8x15		11x27

BULBOCEPHALUS ELONGATUS nov. spec.

[Figures 4, 5, and 6]

Host: *Cucujus* larva (Det. Adam Boving)

Habitat: Intestine

Location: Oyster Bay, Long Island, August, 1916

Specimens of this peculiar gregarine were very abundant in the one beetle larva obtained. The sporonts are relatively very long and slender, unlike those of any gregarine before taken by the writer. The maximum length recorded for a sporont is 600 μ ; the maximum width, 50 μ . The ratio of length of protomerite to total length is 1:11, and the ratio of width of protomerite to width of deutomerite about 1:1.

The protomerite is widest near the septum, at which there is a slight constriction, and the deutomerite is widest at the "shoulder," gradually tapering from thence to form a very long posterior extremity. The protoplasm is dense in the sporont, but much less so at the posterior

end, and the nucleus is obscured in the adult. In the trophozoite, it is seen to be small and ellipsoidal and placed diagonally to the long axis of the animal.

The epimerite is a rather long, stout style dilated in the middle to form a good sized papilla. The shape of the epimerite indicates that this species is closely allied to the preceding, and is therefore placed in the new genus, *Bulbocephalus*.

A table of a few measurements in microns follows:

Length epimerite	x	x	40
Length protomerite (without epimerite). ..	47	30	20
Length deutomerite	553	300	170
Total length sporont.....	600	330	230
Width epimerite	x	x	10
Width protomerite	35	40	20
Width deutomerite	30	50	23
Ratio length prot.: total length.....	1:13	1:11	1:11
Ratio width prot.: width deut.....	1.1:1	1:1.2	1:1.1
Diameter nucleus	5x11

PYXINIA BULBIFERA nov. spec.

[Figures 7, 8, 9, 10, 11 and 12]

Host: *Dermestes lardarius* Linn. (Det. E. P. Felt)

Region of infection: Mid-intestine

Location: Oyster Bay, Long Island, May, 1916

Fifty or more specimens of this parasite were taken from one adult larder beetle, many of them being free trophozoites.

The sporonts are solitary and long and slender in shape, except at the "shoulder," where they are appreciably wider (Figs. 7 and 8). The largest sporont seen measured 850μ long and 160μ wide; the smallest specimen without an epimerite, 600μ long. The average ratio of length of protomerite to total length of sporont is 1:5, and the ratio of width of protomerite to width of deutomerite is 1:1.3.

The protomerite is generally of the same width as height, infrequently a little wider than high. It is bluntly cone-shaped, the widest portion lying two-thirds the distance from the apex, being constricted below this to meet the septum. There is often a slight indentation at the apex, left by the dropping off of the epimerite. The epicyte forms a very thick layer over the end of the protomerite (Fig. 9).

The epimerite consists of two parts, a stout bulbous crenulate and crateriform base and a short thick style. The bulbous base was frequently seen to expand and contract with considerable force by means of longitudinal folds which open and collapse, the motion of the myonemes probably forcing protoplasm in and out of the epimerite from the protomerite (Figs. 10, 11 and 12). The epimerite measures from 60μ to 100μ in length on large free trophozoites. The largest epimerite seen measured as follows: Style, 70μ long and 10μ wide at its base; bulb 30μ high and 35μ wide at its widest part.

The deutomerite tapers gradually from the "shoulder," ending in a blunt point.

The protoplasm is dense in most parts of the body. The widest portion of the deutomerite is very dark brown, nearly black, the posterior end being lighter in color. The protoplasm gradually becomes less dense in the latter region and the granules cease entirely some distance before the end is reached. The protomerite is tan in color and the epimerite transparent.

The nucleus is scarcely visible in an unstained sporont, but in stained specimens is seen to be an ellipsoidal body lying always at right angles to the main axis of the body and never diagonally, as is often the case in gregarines. A single large karyosome is present. The nucleus measured in a trophozoite about 90 by 40 μ .

Myonemes were visible at the end of the deutomerite and in the protomerite with a magnification of 490; longitudinal striations were seen with a magnification of 770 and intense transmitted light. Brownian movement was noted where the protoplasm was least dense, viz., at the apex of the protomerite and the tip of the deutomerite.

Neither cysts nor spores were seen.

Although species of the genus *Pyxinia* have been described from the genus *Dermestes*, the present specimens do not fit any of these descriptions. They differ from *Pyxinia rubecula* Hammerschmidt (Léger, 1892: 140) chiefly in shape of the epimerite, which in the latter species is urn-shaped with a wide mouth and a crenulate periphery, and with a short style. Léger's figure for an epimerite of this species indicates the diameter of the urn to be five times its depth and the minute style so short that it scarcely projects beyond the rim. The present specimens indicate an urn narrower at the periphery than elsewhere and a style nearly as wide at its base as the encircling crenulate rim. In shape and proportions, the two species compare favorably, and the host is a beetle of the same genus, although previously described from Europe.

The conspicuous refractile pyxinin crystals of *P. crystalligera* Frenzel (1892: 314) are lacking in the present species, and the long, slender sporonts with bulbous protomerites contrast strongly with the species described above. Maximum length of sporonts and shape of the epimerite are similar in the two.

The other two described species of *Pyxinia*, *P. frenzeli* Laveran and Mesnil, and *P. möbussi* Léger and Duboscq (Watson, 1916: 151) are radically different from this species in size and shape of the sporonts and in the character of the epimerite.

Dimensions of a number of typical specimens are appended herewith, measurements being in microns:

Length epimerite	x	x	40	x	100
Length protomerite (without epimerite) ..	150	150	150	160	140
Length deutomerite	700	690	600	580	500
Total length	850	840	750	740	640
Width protomerite	150	150	140	110	120
Width deutomerite	160	200	190	150	150
Ratio length prot. (without epim.):					
total length	1:5.6	1:5.6	1:5	1:4.6	1:4.6
Ratio width prot.: width deut.....	1:1	1:1.3	1:1.3	1:1.3	1:1.2
Diameter nucleus					90x40

GREGARINA NEGLECTA nov. spec.

[Figures 13, 14, 15, and 16]

Host: *Ceuthophilus neglectus* Scudder (Det. A. N. Caudell)

Habitat: Intestine

Location: Oyster Bay, Long Island, August, 1916

Four associations of this gregarine were found in the mid-intestine of one specimen of this camel cricket. Although very similar to many other species of the genus *Gregarina* in shape, this species may be characterized by a papillate protomerite in the primate and a perfectly egg-shaped satellite, there being no constriction at the septum.

The associations seen were all of about the same length, the maximum length of an association being 900μ ; that for a single-sporont 500μ . The maximum width measured was 230μ . In every instance, the primate was longer than the satellite, maximum length of the satellite being 430μ . The average ratio of length of protomerite to total length of the primate is 1:6; for the satellite the average is about 1:8.

The primate is elongate-ellipsoidal in shape and the satellite ovate, widest anteriad. The protomerite of the primate is broadly rounded, widest at the base and papillate at the apex; it is approximately one and two-thirds times as wide as high. There is a slight constriction at the septum. The deutomerite is regularly ellipsoidal, terminating in a broadly rounded extremity.

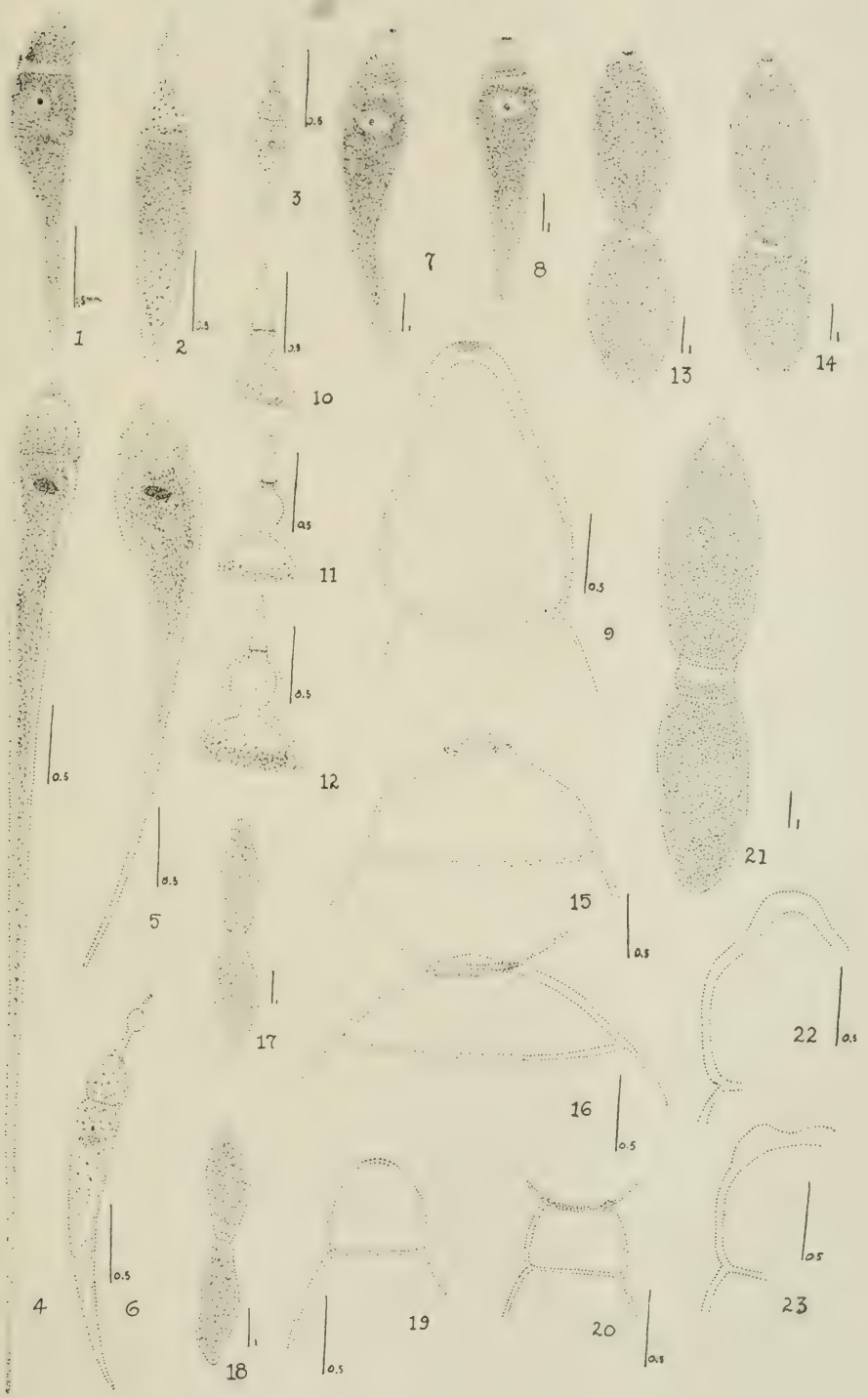
The protomerite of the satellite is very broadly rounded anteriorly, but with a trace of the papilla so prominent in the primate. It is three times as wide as high and is widest at the septum; there is no constriction at the septum. The deutomerite is widest near the middle and terminates in an extremity less well rounded than in the primate.

This species is very dark brown in color with the protomerite slightly less dense. In the satellite, both protomerite and deutomerite are very dark, rendering the septum difficult to trace. The nucleus is completely obliterated in the living animal. The epicyte is very thin, much less so than is usual with this genus.

EXPLANATION OF PLATE

- Fig. 1.—Sporont of *Bulbocephalus wardi*.
Fig. 2.—Large free trophozoite of *B. wardi*, with epimerite.
Fig. 3.—Smaller trophozoite of *B. wardi*.
Figs. 4, 5.—Sporonts of *Bulbocephalus elongatus*.
Fig. 6.—Free trophozoite of *B. elongatus*.
Figs. 7, 8.—Sporonts of *Pyxinia bulbifera*.
Fig. 9.—Protomerite of *P. bulbifera*, to indicate thickening of epicyte and indentation left by epimerite.
Fig. 10.—Epimerite of *P. bulbifera*, not expanded.
Fig. 11.—Epimerite of *P. bulbifera*, with bulb partially expanded.
Fig. 12.—Epimerite of *P. bulbifera*, bulb fully expanded.
Figs. 13, 14.—Two associations of *Gregarina neglecta*.
Fig. 15.—Protomerite of primate of *G. neglecta*, to indicate papillate apex and thickening of epicyte.
Fig. 16.—Protomerite of satellite of *G. neglecta*.
Figs. 17, 18.—Two associations of *Gregarina polymorpha*.
Fig. 19.—Protomerite of primate of *G. polymorpha*.
Fig. 20.—Protomerite of satellite of *G. polymorpha*.
Fig. 21.—Association of *Gregarina blattarum*.
Fig. 22.—Protomerite of primate of *G. blattarum*, to show thickening of epicyte at apex.
Fig. 23.—Protomerite of satellite of *G. blattarum*.

PLATE



Cysts were found in three stages of development, measuring in internal diameter about 300μ (see table of dimensions). No spores were seen.

Gregarines have been described from the genus *Ceuthophilus* in three cases: two by Ellis, and one by myself (Watson, 1916: 114-5). The present species is differentiated from the first two species in shape, from the one in shapes of the two protomerites in the association, from the other in shapes of the deutomerites. It differs from the last described species in size of sporonts and cysts and in relative thickness of the epicyte, the associations of the present species being approximately three times the length of those of the species formerly described, and the cysts twice as large in the present species (150μ and 300μ in diameter, respectively).

A table of measurements is appended, dimensions being stated in microns:

	Primate				Satellite			
	a	b	c	d	a	b	c	d
Length protomerite	70	100	70	70	50	40	50	60
Length deutomerite	430	400	390	400	350	330	370	370
Width protomerite	130	150	120	120	170	190	130	150
Width deutomerite	210	230	210	190	200	210	200	200
Total length sporont....	500	500	460	470	380	370	420	430
Ratio length prot.: total								
length	1:7.1	1:5	1:6.5	1:6.7	1:7.6	1:9.2	1:8.4	1:7.1
Ratio width prot.: width								
deut.	1:1.5	1:1.5	1:1.7	1:1.6	1:1.2	1:1.1	1:1.5	1:1.3
Total length association.	880	870	880	900				
Diameter cysts:								
Without envelope...	300	310	290					
With envelope.....	none	470	none					

GREGARINA POLYMORPHIA (Hammerschmidt) Stein

[Figures 17, 18, 19, and 20]

Host: Larva of a Tenebrionid, probably *T. molitor*

Region of infection: Intestine

Location: Oyster Bay, Long Island, August, 1916

One specimen of this Tenebrionid beetle larva from a meal sack was found to contain half a dozen associations of one of the gregarines.

The specimens are characterized by a regularly curved protomerite in the primate and a protomerite of the same shape, but slightly flattened, in the satellite.

The sporonts vary in length from 280μ to 320μ , in width from 100μ to 120μ . The ratio of length of protomerite to total length of sporont is approximately 1:7 for both primate and satellite; the ratio for width of protomerite to width of deutomerite is about 1:2.

The sporonts are small, elongate ellipsoidal with a relatively small protomerite. The protomerite is beautifully rounded in outline in the primate, representing about two-thirds of a sphere. The widest part

is just below the middle, and there is a distinct constriction at the septum. The protomerite of the primate is approximately as wide as high; while that of the satellite, which is the same shape but flattened at the apex, is wider than high. The deutomerite is widest at about its mid-region, tapering anteriorly to the septum and posteriorly, ending in a long blunt cone.

The protoplasm in the sporont is abundant, rendering it dark brown, almost black in the deutomerite and dark tan in the protomerite. No nucleus is visible in either member of the adult association. The ectosarc is especially thin, even at the tip of the protomerite and at the septum, causing rupture to be easily effected in water, an unnatural medium.

The animals are relatively active in movement, both of progression and contortion.

Neither cysts nor spores were seen.

This species has been described from Europe and Japan, but not heretofore from the United States. The first mention of the species was made in 1838 by Hammerschmidt, and it has been seen at various times since then, by Stein, Frantzius, Schneider, Léger and Duboscq, Ishii, etc.; see Watson, 1916:172, for correlations and references. No two descriptions and drawings tally exactly, but there is not sufficient evidence to differentiate the observations into separate species. The present detailed description for a known species is given because here also are indicated variations from the original of Hammerschmidt, and since the specimens seen by every worker differ slightly from those of the others, it does not seem wise even to describe a new variety.

A table of a few typical measurements follows, dimensions being in microns:

	Primate				Satellite			
	a	b	c	d	a	b	c	d
Length protomerite	60	40	40	40	40	40	40	40
Length deutomerite	250	270	240	280	280	280	240	270
Width protomerite	60	50	50	40	70	60	40	60
Width deutomerite	110	110	100	120	120	110	100	120
Total length sporont....	310	310	280	320	320	320	280	310
Total length association.	630	630	560	630				
Ratio length prot.: total								
length	1:5.1	1:7.7	1:7	1:8	1:8	1:8	1:7	1:7.7
Ratio width prot.: width								
deut.	1:1.8	1:2.2	1:2	1:3	1:1.7	1:1.8	1:2.5	1:2

GREGARINA BARBARARA Watson 1915

This species was found at Oyster Bay, Long Island, in *Adalia bipunctata* in June, 1916. This, our commonest lady beetle in the East, was examined frequently throughout the year, but was found parasitized but once. Two associations and half a dozen isolated sporonts

were found. This species has heretofore been recorded from an unidentified *Coccinella*, not the present species.

A few measurements, in microns, are appended to supplement those already given (Watson, 1915:31 and 1916:185), and for sporonts somewhat smaller than those previously described.

	Primate				Satellite
	a	b	c	d	a
Length protoimerite	20	30	20	20	8
Length deutomerite	80	70	70	80	52
Width protomerite	30	30	30	30	30
Width deutomerite	55	60	45	55	40
Total length sporont	100	100	90	90	60
Total length association	160				
Ratio length prot.: total length	1:5	1:3.3	1:4.5	1:5	1:7.5
Ratio width prot.; width deut.	1:6	1:2	1:1.5	1:1.6	1:1.3
Diameter nucleus	12			

GREGARINA BLATTARUM Siebold

[Figures 21, 22, and 23]

Host: *Blatta orientalis* Linn

Regions of infection: Intestine and rectum

Location: Urbana, Illinois, June, 1915

A dozen or more biassocative gregarines were found in one specimen of the Oriental cockroach, this being the greatest number found by the writer in a single host. Many roaches were examined with negative results.

The insects are also parasitized by two species of nematodes, an infusorian, and an amoeba, the last two of which were described by Leidy in 1877 and 1881.

This gregarine is characterized by long, slender sporonts, blunt at the posterior end, by a conical, or papillated, protomerite in the primate, and a broad, flattened protomerite in the satellite.

The sporonts vary in length from 510 to 1100 μ , and in width from 160 μ to 400 μ . The average ratio of length of protomerite to total length of sporont is 1:5 for the primate and 1:8 for the satellite. The protomerite of the primate is approximately two-thirds as wide as the deutomerite, that of the satellite approximately three-fourths to fully as wide as the deutomerite. The ratio in the primate of width of protomerite to width of deutomerite is about 1:1.7, in the satellite about 1:1.4.

A table of representative measurements is appended herewith.

The protomerite of the primate is bluntly pointed, the ectoplasm at the apex being a much thicker layer than elsewhere in the animal. The widest portion of the protomerite is about two-thirds of its length from the apex, and there is a slight constriction at the septum separating protomerite and deutomerite.

The deutomerite is elongate ellipsoidal, varying but little in width throughout the length, and broadly rounded at its posterior end. The end attached to the satellite is but little flattened.

The protomerite of the satellite is slightly flattened anteriorly, and there is but little or no constriction at the septum. The deutomerite is more or less irregularly shaped, ending in a rather blunt point.

The nucleus is small and spherical. It measures about 90μ in diameter in sporonts. The spherical karyosome is faintly visible.

The deutomerite of the sporonts is very dense and blackish, the protomerite slightly less dense and dark tan in color. The deutomerite is finely granular and homogeneous, and is slightly more dense in the satellite than in the primate. The nucleus was not visible in the satellite of any specimen seen. The protomerite contains large spherical masses less closely packed together than in the deutomerite.

This species was found first by Siebold in Germany, and by many subsequent workers, including Frantzius, Leidy, Schneider, Marshall, de Magalhaes, and Crawley, and from Germany, France, Brazil, and Pennsylvania (see Watson, 1916: 99-100).

The present specimens have the same general proportions as those already described, but reach a much greater length than that stated by Leidy, which is 500μ for a single sporont; no other writer has given dimensions. The specimens now described are undoubtedly closely related to the species he saw and described as *Gregarina blattae orientalis*, for he mentions the "slight papillary thickening of the integument" at the apex of the protomerite and indicates this feature in his three drawings. He does not state, however, whether or not the species is associative.

Because of the very considerable confusion surrounding this classical species in the past, it does not seem to the writer wise to add to it and describe the specimens now found as a variety of the type species when the only disparity is found in a papillate or non-papillate apex of the protomerite. A number of variations have already been described, but are now separated into species.

The measurements given below are in microns.

	Primate				Satellite			
	a	b	c	d	a	b	c	d
Length protomerite	120	150	160	200	60	80	100	160
Length deutomerite	390	720	790	900	460	750	600	870
Width protomerite	120	160	200	200	150	160	250	250
Width deutomerite	200	260	300	360	200	230	250	400
Total length sporont.....	510	870	950	1100	520	830	800	1030
Ratio length prot.: total								
length	1:4.2	1:5.8	1:5.9	1:5.5	1:8.6	1:10	1:8	1:7
Ratio width prot.: width								
deut.	1:1.7	1:1.6	1:1.5	1:1.8	1:1.3	1:1.4	1:1	1:1.6
Diameter nucleus		90						
Total length association.	1030	1700	1750	2130				

A new place record has been established for *Leidyana erratica* (Crawley) Watson, at Douglas Lake, Michigan, by Mr. H. G. May, of the University of Illinois. This species and *Gregarina oviceps* Diesing were very commonly parasitic in the same host, which has been designated *Gryllus americanus* Blatch. Mr. May says, however, that the host may be *G. pennsylvanicus* Burm., or even an hitherto undescribed species of cricket. The cricket fits neither description perfectly.

In none of the instances recorded above was it possible to complete a life-history of the parasite because the hosts are for the most part uncommon and rarely more than one specimen of the same species was taken during the summer. It was unfortunately impossible to secure any intestines for sectioning purposes and, as cysts were rarely seen in the host parasitized, the life-histories were not carried to completion.

Type specimens of the above species have been deposited in the Ward Collection of Parasites at the University of Illinois.

SUMMARY

A new genus, *Bulbocephalus*, with two new species is described for the family *Stylocephalidae*.

New species are described for *Pyxinia* and *Gregarina*, and new data are given for *Gregarines* already known.

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ON A TREMATODE LARVA ENCYSTED IN A CRAB,
HELICE TRIDENS (DE HAAN)

SADAO YOSHIDA

Pathological Department of Osaka Medical Academy

In November, 1915, a considerable number of the encysted larvae of a trematode and some specimens of an infected crab were forwarded for identification by T. Urita, a friend of mine in Kagoshima, with a letter which read as follows: "The liver and the inner surface of carapace of a crab, *Helice tridens* (de Haan), are heavily infested with the egg-like cysts with thick, transparent wall containing a moving worm which seems to be a trematode larva. Some worms creep out. The crabs are found abundantly in the salt-field living in the holes they dig. Seventy to ninety per cent or more among the crabs in the vicinity of Kagoshima City are infected with the cysts."

Afterwards I obtained the crabs of Kagoshima Prefecture on several occasions from the same friend and from H. Yamakado, a student of our academy, and collected them myself on the seashore near Osaka. The crabs are found in various districts, especially in the warm parts of this country. They live in ground near the seashore, such as salt-fields, river mouths, and other coast areas exposed when the tide ebbs, and habitually build burrows in which they live. The crabs are not used as food in Japan proper, but they are said to be eaten in some parts of Korea and Formosa. There is no need to describe the morphological characters of the crab here.

On several occasions I examined two hundred and fifty specimens of the crabs and found about 90 per cent of them infected with the cysts. There is no local difference in frequency of infection between the crabs collected near Osaka and those of Kagoshima. The number of cysts harbored in a crab is variable but generally large, varying from one to several hundreds, though sometimes they are in masses by the thousand. The encysted larvae occur generally in the ovary, on the wall of the stomach, in the hypodermis lining the carapace, and other parts of the body cavity of the host. The ovary (Fig. 1A) and hypodermis are most heavily and frequently infested with them. In the liver I have rarely found the cysts, although T. Urita informed me of the liver infection in his letter quoted above. I found occasionally, however, infected pieces of membranous tissues twining round the lobes of the liver, a condition which perhaps would suggest liver infection.

Morphology of the Encysted Larvae.—The encysted larvae found in the crab are oval or rarely spherical in shape, varying more or less in size. Measurements of eight cysts show a range in length from 0.407 mm. to 0.601 mm., and in breadth from 0.366 mm. to 0.510 mm. The average length was 0.521 mm. and breadth, 0.418 mm.

A slight pressure by the cover-glass may alter the dimensions of the cysts; the measurements given above are obtained from those not compressed.

The wall of the cyst is a transparent chitinous membrane from 0.02 to 0.04 mm. thick through which the larva may be readily observed. An actively moving larva in the fully developed cyst is light brown in color with dark spots marking the position of the yolk glands. The worm occupies almost all the space within the cyst with the body bent in various fashion (Figs. 1 *B, C*). In the most common position in the cyst, either the anterior or posterior extremities of the body, or both, coil ventrad, and both lateral margins of the anterior region are also sometimes bent over the ventral surface. Through the wall of the cyst one may recognize the organs of the larva, i. e., oral sucker, alimentary tract including pharynx, long esophagus, intestinal ceca, excretory vesicle, and such genital organs as ovary, yolk glands and testes; but the identification of these organs is complicated by the movements of the body.

The encysted larvae in full development may emerge from the cysts at various times after the latter are removed from the crab and put in distilled water or physiological salt solution. Sometimes this takes place within only ten to thirty minutes after the cysts are removed from the host. Cysts from dead crabs are generally somewhat weakened, in consequence of which the larva may easily emerge from the cyst. Encysted larvae from crabs that have been dead a long time are also dead or so strongly affected as to be at the point of death. It is believed that the action of the putrescent fluids explains the fact that the larvae are generally found out of the cyst and dead. During my study I often found the larvae free and dead after a day or two in culture dishes.

External Feature of the Larva Liberated from the Cyst.—The larva freed from a fresh cyst is actively mobile, changing its shape and size greatly, especially in the anterior region. Figure 2 *B* serves to show the extent of change in the outline of a worm in the contracted state. On the whole, it is concave on the ventral surface and convex on the dorsal; an accurate measurement of length and breadth is very difficult when the worm is alive. The most natural form, however, is to be found in a larva that has recently died a natural death. The larva put between the slide and cover-glass also assumes a form resembling

the natural, though slightly exaggerated. The most common form then assumed is an elongated oval, the middle one-half or two thirds of the body length being of nearly uniform breadth. Both extremities taper, the posterior being more obtuse than the anterior end. In seven fixed specimens measured, the length varied from 0.533 to 0.833 mm., and the breadth from 0.283 to 0.325 mm., the average dimensions being 0.701 by 0.301 mm. Two living specimens measured, respectively, 1.0 by 0.50 mm. and 0.922 by 0.43 mm. Four specimens compressed and mounted in potassium acetate varied from 0.9 by 0.466 mm. to 1.18 by 0.56 mm., with an average of 1.083 by 0.515 mm. Five specimens compressed and mounted in Canada balsam varied from 0.75 by 0.35 mm. to 1.07 by 0.50 mm., with an average of 0.918 by 0.438 mm.

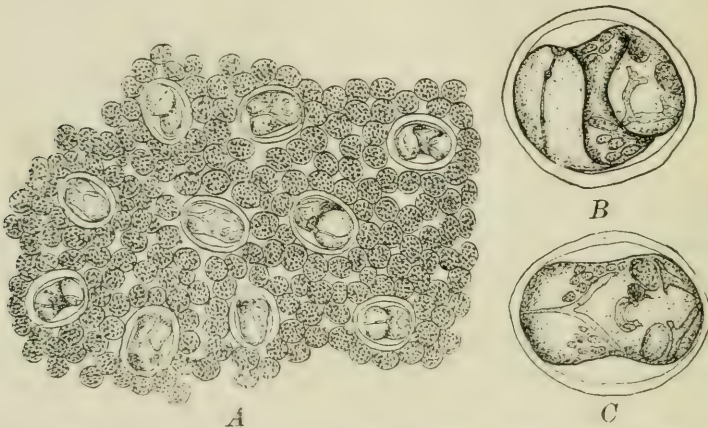


Fig. 1.—*A*, portion of the crab's ovary infested with cysts. $\times 23$. *B*, *C*, two encysted larvae. $\times 60$.

The entire surface of the body is armed with minute spines which are denser in the anterior region than in the posterior and inconspicuous in mounted specimens. The oral sucker (Fig. 2 *B*) is subterminal and spherical or ovoid in shape; its aperture varies according to the state of contraction of the organ. The ventral sucker (*B*) situated about one-third the body length from the caudal end is oval or spherical and smaller than the oral sucker. In the living specimen it is usually indistinct. The average dimensions of the oral sucker in compressed living specimens were 0.053 by 0.05 mm.; in specimens mounted in potassium acetate, 0.0693 by 0.0625 mm.; in specimens mounted in Canada balsam, 0.052 by 0.055 mm. The average dimensions of the ventral sucker in compressed living specimens were 0.037 by 0.024 mm.; in specimens mounted in potassium acetate, 0.035 by 0.040 mm.

Internal Structure.—The internal structure of the larva (Fig. 2) is easily observed in the living specimens compressed or in the mounted

specimens. The larva is remarkable in that almost all the essential genital organs are well developed.

The prepharynx (*P*) is distinct but short and slender, 0.03 to 0.08 mm. long and 0.01 to 0.02 mm. broad. The pharynx (*H*) is sub-spherical, 0.035 to 0.05 mm. long and 0.025 to 0.048 mm. broad. The esophagus (*E*) is very long and slender, 0.25 to 0.35 mm. long, with the breadth gradually increasing posteriorly to a maximum of 0.015 to 0.027 mm. The intestinal ceca (*C*) are short, running from the posterior end of the esophagus obliquely postero-lateral, so as to form a V-shape, the distal ends being far from the lateral margins of the body. The length of the ceca on both sides is nearly equal in the same individual and slightly variable in the different specimens (0.2 to

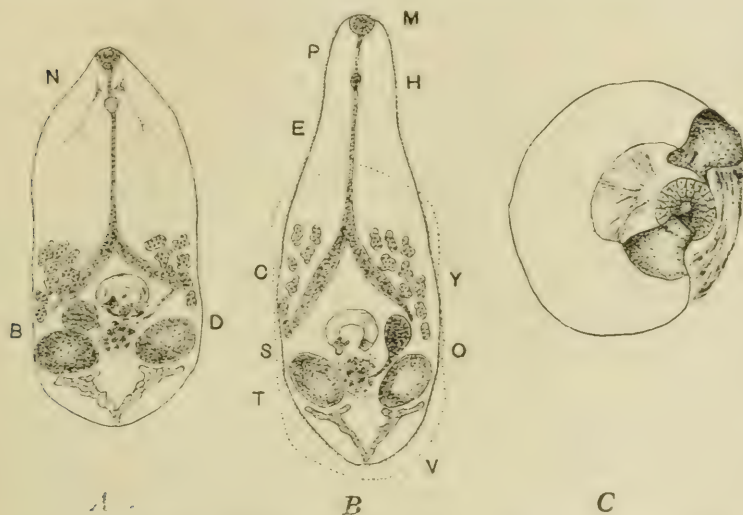


Fig. 2.—*A*, specimen mounted in potassium acetate. $\times 60$. *B*, living larva in motion. $\times 60$. *C*, semilunar organ and ventral sucker. $\times 280$.

0.3 mm. long). The breadth is usually maximum at the middle part of the cecum, but it may be variable according to the condition of its contents.

The excretory vesicle (*V*) is V- or Y-shaped, and each inner end gives off two branches. The winding and branching canals arise from the anterior end of the vesicle, but their entire course cannot be made out distinctly.

The cerebral ganglion (*N*) surrounding the prepharynx and the lateral nerves from it may be observed in good specimens.

Genital Organs.—Two testes (*T*) lie side by side near the posterior end of the body, just in front of the excretory vesicle. They are oval and subequal with the long axes transverse or obliquely to the axis.

They measure 0.15 to 0.26 mm. in length and 0.09 to 0.16 mm. in breadth. The vas deferens arises from the mesial surface of each testis, but its further course cannot be traced. Just anterior to the ventral sucker there is a peculiar organ (*B*) resembling the cirrus sac. It is semilunar, lying transversely with concave side posterior; each extremity is provided with a chitinous process of form unlike the other. The chitinous process on one side (usually the right) tapers from its wide base and terminates in a smaller single tubular end, while the distal end of the process on the other side is divided into smaller unequal shaped processes (Fig. 2 *C*). The free ends of both chitinous processes face each other across the ventral sucker. The significance of this semilunar organ cannot be determined and no connection with other organs was determined. Before and behind the ventral sucker there are muscle fibers connecting with the semilunar organ.

The ovary (*O*) lies on one side (usually the right) of the median line and in the space bounded by the semilunar organ, the posterior end of the intestinal cecum, and the testis on the same side. It is oval or spherical in shape, being 0.07 to 0.12 mm. long and 0.07 to 0.1 mm. broad. The short oviduct springs from its postero-mesial aspect and runs postero-mesial to a cell-group situated in the space between both testes and the semilunar organ. This probably represents the shell gland and the uterus. Here one may find granules very similar when stained to the yolk granules. The cell-group is deeply stained by carmine.

The yolk glands (*Y*) occupy a small area on the antero-lateral aspect of each cecum. The glands on each side consist of from nine to eleven follicles on the ovarian side and from seven to nine on the other side. These follicles are irregular in form and vary slightly in size; but the maximum diameter varies between 0.055 and 0.08 mm. and the minimum between 0.027 and 0.045 mm. The yolk duct (*D*) from the glands runs postero-mesial to the cell-group mentioned above. The genital aperture could not be detected.

From the characteristics mentioned above it is difficult to determine the adult form corresponding to the encysted larvae. I endeavored to find the matured form by animal experiments. On November 26, 1915, many cysts from the crabs were fed to three guinea-pigs, and on December 14 a number of others to two young cats. These animals died or were killed after several days and were carefully examined for distomes, but in vain. On February 5, 1916, several cysts were eaten by three guinea-pigs, but they did not develop. On April 25 cysts were given to five frogs. After four or five days the frogs were killed and examined for the distomes. In the stomach, intestine, and cloaca

larvae were found free from the cysts and dead. Thus far in animal experiments I have not succeeded in obtaining the adult.

These encysted larvae evidently differ from the adult less than in most other cases, because the genital organs are so well developed. The alimentary tract, long esophagus, short intestinal ceca and the position of the yolk glands suggest a relationship to the genus *Brachycoelium*. Other feeding experiments with the encysted larvae are under way at present and will be described later fully with the microscopical structure of the worm.

CYTOLEICHUS PENROSEI, A NEW ARACHNOID PARASITE FOUND IN THE DISEASED LUNGS OF
A PRAIRIE DOG, *CYNOMYS*
LUDOVICIANUS

FRED D. WEIDMAN

On March 28, 1907, a male prairie dog, *Cynomys ludovicianus*, died in the Philadelphia Zoological Gardens with acute bronchopneumonia. The inflammatory condition was recognized at the time of autopsy, the lungs being described as diffusely red, standing out well, their apical parts emphysematous and cut surface showing minute white projecting areas. The parasites forming the basis of this communication were not recognized at this time on account of their extremely small size, being among the smallest of which the writer has found record, and much smaller than the ones which were found here (Weidman, 1915) in the lungs of a monkey and upon birds. The skin bore, especially around the head and shoulders, brownish crusts which were tightly adherent to deeper parts and which, when examined microscopically, were found to contain a fungus.

The gross diagnosis of bronchopneumonia was confirmed microscopically, shown by the presence of numerous red blood cells, frequent clumps of fibrin and moderate numbers of leukocytes in the air sacs, with which a greenish brown granular material, doubtless the excrement of the mites, was intermixed. In addition to the inflammatory disease, the sections showed a high grade of emphysema and bronchiectasis (Figs. 14, 15 and 16).

That articulated parasites were also present was recognized in the microscopical sections which shortly and routinely followed the autopsy in 1907, but it was only during a recent review of prairie dog tissues in connection with another parasite of prairie dogs [*Hepaticola hepatica* (Bancroft, 1893) Hall, 1916] that their arachnoid nature was determined. During this interval (about nine years) the tissue had been preserved in alcohol, precluding experiments on transmission of the infestation and observation of living specimens, but not interfering with staining qualities of sections. These, in favorable cases, submitted parasites showing parts of all four legs (Fig. 15) of one side, thus allowing the diagnosis of arachniasis from microscopic sections alone. They are present in large numbers, almost every section containing at least part of one and generally several, and lie for the most part within air sacs, less often in bronchi. They are surrounded by no special

grade of either acute or chronic tissue reaction such as was found in cases of Wellman and Wherry (1910).

It is impossible to state how common the infestation is in these animals because so few come to autopsy. The beasts rarely die upon the surface, doubtless seeking seclusion under ground when they become sick, there to remain until they die. As a result, only two have come to autopsy in the last eleven years in spite of the numbers always on exhibition, and of these, only the one showed pulmonary parasites.

The material for the determination of the new species was obtained by finely teasing a small portion of the lungs, yielding some fifty or more fully developed specimens and no larvae or ova. Of these, the females were more numerous than the males in the proportion of two to one. They had been fixed in formaldehyd, preserved nine years in alcohol (70 per cent.), and were examined after clearing by glycerin or Farrant's medium. From the proportion of tissue examined to the total lung substance the lungs must have contained from one to several thousand parasites.

THE FEMALE

Females selected at random measured as follows:

Pubescent Females	Ovigerous Females.
0.170 x 0.087 mm.	0.200 x 0.120 mm.
0.185 x 0.085 mm.	0.193 x 0.102 mm.
0.175 x 0.087 mm.	0.185 x 0.105 mm.
0.180 x 0.090 mm.	0.190 x 0.109 mm.
0.170 x 0.100 mm.	0.195 x 0.103 mm.

A female (Fig. 1) observed laterally measured 0.190 mm. in length and 0.094 mm. dorsoventrally.

The body is broadly oval, not quite twice as long as broad, not constricted, and broadest at about the middle.

It bears a dorsal shield of subtriangular form with broadly rounded angles whose base lies at a line between Coxa II anteriorly and apex almost at anus, posteriorly. In the latter region the boundary is not sharply marked because the confines pass so gradually into the general integument, but laterally it is, the shield curving ventrally here for a short distance over the lateral parietes. Anteriorly, it shows sharp separation from the anterior part of dorsum in but few specimens, notably the immature ones. With mature specimens its anterior border appears as an anteriorly directed, more or less gentle slope, and when this is very gentle the dorsum may appear to be covered by a sub-rhomboidal instead of a subtriangular shield with rounded angles. It is possible that these different appearances come about in the following way. It will be observed from the measurements given above that the mature females are of plumper form than the immature (Figs. 2

and 3), doubtless brought about by the development of the reproductive organs which produce an internal pressure resulting in expansion of the parietes. Now the anterior margin of the dorsal shield is indicated not by a ridge, nor by special difference in character of integuments (the integument is of the same character over the whole body), but by a downward curved slope (Fig. 1), the steepness of which will be more and more reduced as the foot becomes elevated by the internal pressure. The progressive reduction of this steepness will, now, with maturation, obscure the anterior margin and consequently give more and more continuity between the shield posteriorly and the anterior dorsal integument.

The dorsum carries but one pair of hairs: long, and projecting from its anterior part close to its lateral margins at a level between Coxae I and II. Dorsal pits are large, inconstant, and observed in but few (four out of fifty) specimens. Of these the dorsal shield carries two rows at the level of Coxa III; a more anterior one consisting of two pairs of well outlined pits and an inconstant lateral one, and a more posterior row of but one pair, one pit on each side. The anterior portion of the dorsum shows seven, arranged in two irregular longitudinal rows (Figs. 2 and 3).

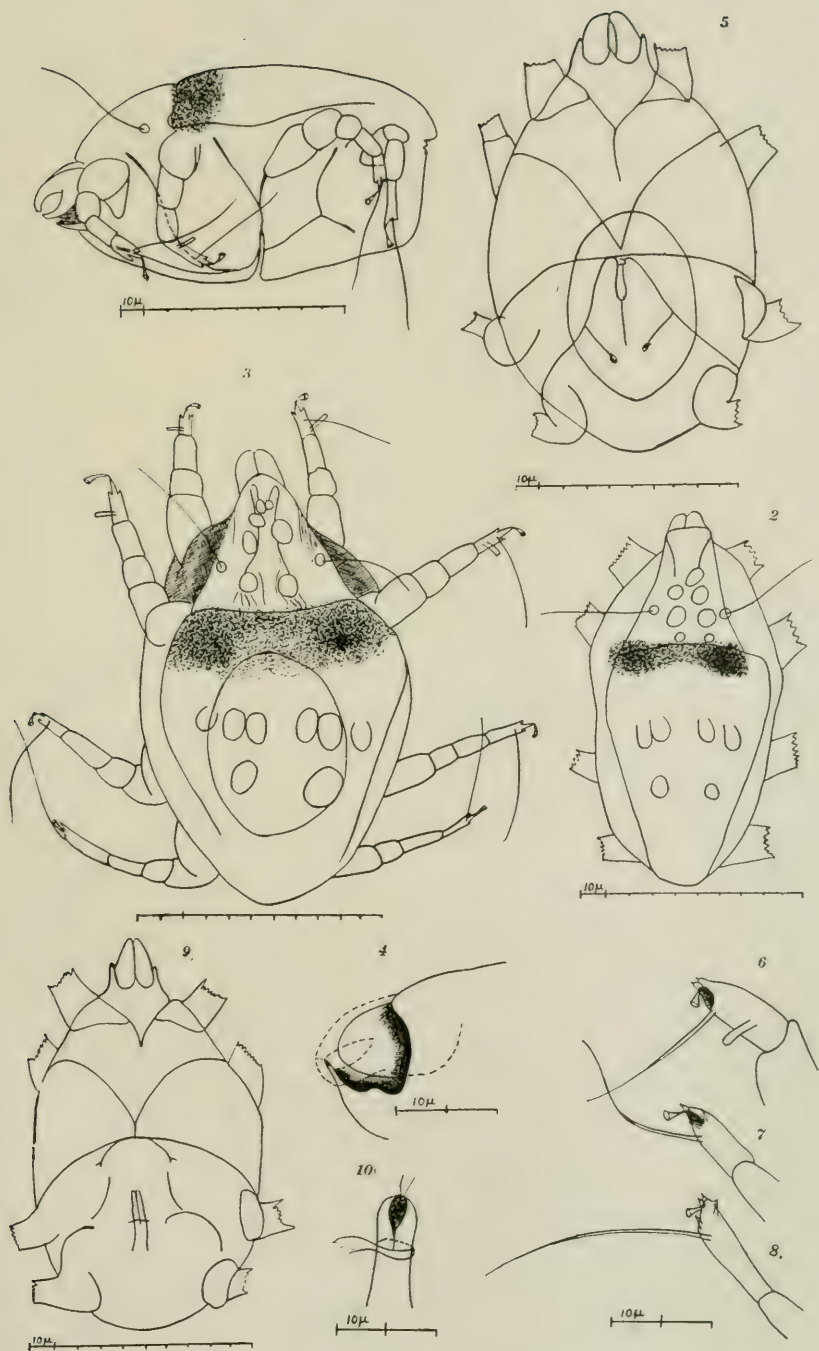
There are no eyes. The epistome is rounded anteriorly and largely covers the mouth parts as viewed dorsally. Laterally, it curves ventrally to become continuous with the hypostome, with which it forms a large tube holding the mouth parts. The hypostome shows two deep lateral scallops, with intervening median peak extending well anteriorly.

Mouth parts project but slightly beyond the body, being deeply retracted, the palpi and chelicers so closely compacted as to make their morphology indeterminate. It cannot be stated how many joints the former have, but it is most probable that they bear no hairs since some, if present, would project and be recognized in the many specimens studied. In rare cases the mandibles are recognized with untoothed chelicers (Figs. 1 and 4).

The dorsal and contiguous lateral integuments are covered by very fine, refractile, granular elevations and show no special linear markings.

The venter is divided, like that of *Sarcoptes scabiei*, into several irregular triangular areas by shallow furrows which are probably of the nature of synarthroses. They begin near the body middle, radiating laterally and curving dorsally, some nearly to the dorsal shield and all ending close to one of the coxae. (For details see Figs. 1 and 5.) The integument here, as with the dorsum, is covered by extremely fine refractile elevations of variable size, shows no linear markings, and appears to be of a soft leathery rather than chitinous nature.

PLATE 1



EXPLANATION OF PLATE 1

Fig. 1.—Lateral view of female.

Fig. 2.—Dorsum of pubescent female.

Fig. 3.—Dorsum of ovigerous female. Coxae of Legs I hidden.

Fig. 4.—The rostrum, seen ventro-laterally. Dotted lines indicate the chelate mandible seen at a lower level through overlying parts. Mandible folded in joints toward mid-line.

Fig. 5.—Venter of ovigerous female.

Fig. 6.—Tarsus of Legs I and II. The seta is foreshortened.

Fig. 7.—Tarsus of Leg III.

Fig. 8.—Tarsus of Leg IV.

Fig. 9.—Venter of male.

Fig. 10.—Male external parts.

EXPLANATION OF PLATE 2

Fig. 11.—Pubescent female. From teasings.

Fig. 12.—Ovigerous female. Also from teasings. The rounded, lighter and darker bodies are red blood cells, the darker material lung fragments.

Fig. 13.—Male. From teasings.

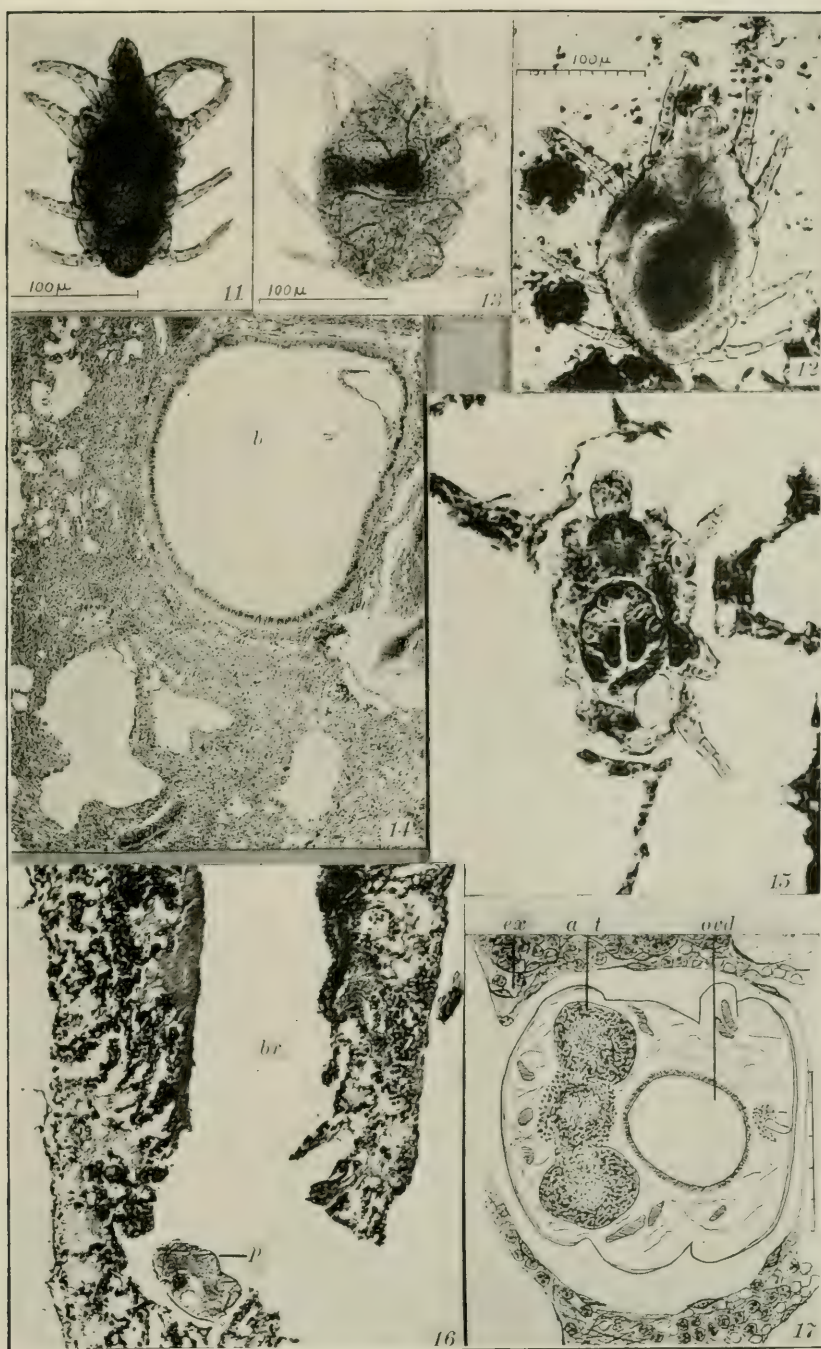
Fig. 14.—Very low-power view of microscopic section of lung showing dilatation of bronchus at *b* (bronchiectasis), irregular distention of some air sacs, and broncho-pneumonic exudate in others.

Fig. 15.—Very high-power view of microscopical section of lung showing longitudinal section of ovigerous female. Note distention of air sacs—emphysema.

Fig. 16.—Low-power view of microscopic section of lung showing parasite (*p*) in lumen of bronchus (*br*).

Fig. 17.—Transverse section through parasite in lung. *at*, alimentary tract; *ovd*, oviduct; *ex*, inflammatory exudate in lung. Each subdivision of scale equals 10 microns.

PLATE 2



The first pair of legs lies close to but not fused with the capitulum. There is a broad interval between it and the second pair, and a broader one between it and the last two pairs. The latter are separated from each other by about the same distance as the first two. As fixed, most of the specimens show the first pair directed anteriorly and the other three strongly flexed upon the coxæ and directed laterally and anteriorly, as shown in Figure 2. With many, however, the legs are flexed toward the middle of venter (Fig. 1), in which case the latter generally pouts, hill-like, strongly ventrally. The first pair is the shortest, measuring 0.07 mm.;¹ the other three but little longer, measuring 0.08 mm., and none of the four is as long as the body width. Each is composed of five segments, distinctly more slender in the last two legs than the first two, particularly the tarsus of Leg IV, which is at least four times as long as broad.

Only the tarsus bears special cuticular appendages. In the case of *all* four legs it bears the following: First, a long hair passing from a point well short of its distal extremity; second, three or four² minute, short, terminal spurs, the median one or two mucronate and a little in advance of the others; third, a small, delicate caroncle extending from the base of the median terminal spur. With Legs I and II only, a short, stout, rod-like seta (sense-hair) extends in addition from a point near its base (Figs. 3 and 6). Legs III and IV do not bear this structure.

The anus is terminal, at junction of dorsum and venter, and surrounded by no special appendages. Stigmal plates in spite of careful search of the abundant material, could not be found.

The vulvar orifice is ventral, median, fissural, longitudinal, lies at level of Coxa III, and ends anteriorly at the arthrosis which passes transversely at about the body middle. No ova were observed free. A specimen in the body of a female measured 80 by 65 μ .

The only internal organ recognizable through the cuticle is part of the alimentary canal, the approximate position of which is indicated by thickly placed, black or brownish-black, fine to coarse granules probably consisting of altered blood pigmentary material (hemoglobin?) which has been ingested as food. It appears over a variable area extending through a greater part of the body width under the anterior

1. All measurements from an ovigerous female 0.200 mm. long unless otherwise noted.

2. The borders of the tarsus become membranaceous at the extremity and appear to be capable, either naturally or artificially, of being folded centrally. This may superimpose the central mucronate spurs so that they appear one, but in numerous cases this possibility can surely be ruled out. We are working here under very high magnification ($\frac{1}{12}$ in. oil immersion lens) where it is often difficult to translate optical appearances into positive statements.

third of the dorsal shield, and at times for a short distance beyond it anteriorly, or farther posteriorly, on each side. Transverse sections of the parasite in lung show two lateral longitudinal tubes hugging the dorsum closely which are evidently intestine, and a single larger ventral one which is probable the oviduct (Fig. 17).

THE MALE

The male differs from the female only in its slightly smaller size, minor differences in configuration of the posterior ventral synarthrosis, and its special genital organs. The measurements of several selected at random follow:

0.175 x 0.102 mm. (Fig. 8)
0.162 x 0.109 mm.
0.170 x 0.100 mm.
0.160 x 0.105 mm.
0.155 x 0.108 mm.

The genital orifice lies ventrally, median, at the level of Coxa III, is transverse, small as compared to its female counterpart, and bordered by a narrow chitinous rim. In most cases the copulatory apparatus projects from it in an anterior direction in the form of a short heavy cylindrical piece with rounded ends. The parts are so homogeneous that finer details cannot be asserted with certainty, but it appears as though the piece were a tube whose wall is split longitudinally through its whole length anteriorly, and whose lumen contains two extremely delicate, curved, sharp-pointed spicules (Figs. 9 and 10). For differences in configuration of posterior ventral synarthrosis compare Figures 5 and 8, a female and male.

PATHOGENICITY

Since knowledge of the clinical course of the disease is lacking, postmortem findings furnish of course the only basis for judging the part played by the parasite in producing death. By studying these, it was found that all of the lesions above described were acute ones, best seen in the microscopic sections, where among other changes emphysema and bronchiectasis were described, both of which are commonly produced by severe coughing.

Now, these two changes may be caused by either acute or chronic coughs. In those cases where the cough is chronic, lasting, say, several months, it is found that fibrous overgrowths occur in addition, particularly in the walls of bronchi, and that infiltrates of lymphocytes are also sometimes associated. But none of these are seen in this case. Bronchial walls are uniformly thin, and free of cells other than those which can be explained by the nearby acute inflammation. There

exists here the *acute* forms of bronchiectasis (or bronchiolectasis) and emphysema (so-called).

It has been already noted that no ova or larva were found in the abundant material studied. It may be added that there is no important difference in size between the mature specimens. These observations, together with the lack of *chronic* pulmonary tissue changes, lead to the belief that the mites were present but a short time, certainly not long enough to reproduce, and so probably not longer than a few weeks, as *Sarcoptes scabiei* matures from the larva in about three weeks. Under these circumstances it is the reasonable thing to believe that it is the parasites which have excited the acute bronchopneumonia and so induced death.

The two cases of Wellman and Wherry concerned parasites of squirrels which were well encapsulated in tubercular nodes, i. e., had been present for some time. It is possible that the squirrels, too, had suffered from an acute attack of bronchopneumonia at the time of infestation, and if this be true for them, it should also be thought that the disease produced by *C. penrosei* may also be recovered from at times and the mites encapsulated.

The original source and mode of entry are only speculative, as discussed in the case of the monkey infestation which has been referred to earlier (1915) as occurring in these gardens.

ZOOLOGICAL POSITION

Following Banks' (1905) key, this is indicated as follows: Class, Arachnoidea; order, Acarina; superfamily, Sarcoptoidea; family, Cytoleichidae. He describes the family as consisting of two species, *Cytoleichus* (formerly *Cytodites*) *nudus*, and *Laminosioptes cysticola*, and gives family diagnosis as "In skin and cellular tissue of birds. Vulva longitudinal." He does not include in this family the original type species, *C. sarcoptoides*, Megnin, 1879, perhaps on account of the scope of his paper, or perhaps because the species has been later placed in another genus. Nor does he give the generic diagnosis of *Cytoleichus*, which I assume to be still extant, as reproduced as follows from Megnin (1879):

"Body large, orbicular, convex above, plane below, continued anteriorly by a mobile, inclined, conical, tubular rostrum covered above at its base only by an epistome provided with no appendages like joints, etc. Legs conical, robust, arranged in two groups, a cephalothoracic and an abdominal, the first only being marginal, the epimerae of the first pair alone fused to form a sternal plate, the others free; tarsi without terminal hooks, only a ventral simple ambulacrum with cylin-

drical pedicle; the tarsus of the second pair shows at all ages in both sexes a blunt cirrus directed above and outward. Ovo-viviparous acarians. Type species, *C. sarcoptoides*. Habitat, air sacs of birds (pheasants)."

Comparison of the above diagnosis with *C. penrosei* shows several important differences, in spite of which the writer has placed this parasite in the genus *Cytoleichus*, mainly because it resembles the mites placed there by Wellman and Wherry (1910) as *C. banksi*. They were found in large numbers in the lungs of two California ground squirrels (*Otospermophilus beecheyi*), each within a tubercle, and occurred both on and within the lung substance. Their description is a brief one, and so far as it goes agrees fairly with this prairie dog species, but their illustration while a simple one, is of value here in that it shows (1) the joints of the last pair of legs distinctly heavier than in the prairie dog species, and (2) the intestinal markings far posterior. The type specimen is not available for original reference since it is recorded as in the collection of Creighton Wellman, who cannot be located in spite of some correspondence. From the data at hand, *C. banksi* would seem to differ from *C. penrosei* mainly in that (1) the joints of the posterior legs are thicker; (2) it bears no short sense-hairs upon the first two pairs of legs, or (3) longer ones upon the dorsum, and (4) no dorsal shield is mentioned. It is not possible that the two prominent dorsal hairs could have been overlooked by Wellman and Wherry (1910) had they been present in the squirrel species, nor scarcely the sense-hairs on Tarsi I and II; but it is quite possible that the dorsal shield might not be considered an entity by some observers. These differences determine a new species, *C. penrosei*.³

The writer feels that *C. banksi* and *penrosei* collectively show wide enough differences from the type to warrant the construction of a new genus to include them. Thus, *C. sarcoptoides*, the type, lives in birds, the other two in rodents; *C. sarcoptoides* measures nearly three times as large (0.570 mm. by 0.440 mm.), and does not bear the long tarsal seta common to *C. banksi* and *penrosei*. He prefers, rather, not to multiply genera, but to leave this to some systematist who will study a larger group of species than the occasional medical writer.

C. penrosei nov. spec. Specific diagnosis: Grossly invisible, broadly oval, with dorsal shield and bearing one pair of long hairs anteriorly. Legs nearly equal in length, none longer than body width, each with five joints, the tarsus of each with long hair near and delicate caroncle at tip. Tarsi of Legs I and II with short stout sense-hair near base

3. Dedicated to Dr. Charles B. Penrose, the president of the Philadelphia Zoological Society.

in addition. Vulva median, longitudinal, fissural, between Coxa III. Male genital orifice in similar position, but transverse. Females average 0.193 by 0.108 mm., males, 0.164 by 0.105 mm.

Habitat, lungs of prairie dog, *Cynomys ludovicianus*.

Type specimen in Philadelphia Zoological Gardens. Autopsy No. 1044.

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1915a. An Arachnoid (*Pneumotuber macaci* Landøis and Hoepke?) Parasitic in the Lungs of a Monkey (*Macacus rhesus*). Jour. Comp. Path. and Therap., 28: 326-330.

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BOOK REVIEW

MEDICAL AND VETERINARY ENTOMOLOGY. William B. Herms. A textbook for use in schools and colleges as well as a handbook for the use of physicians, veterinarians and public health officials. New York: The Macmillan Company, 1915. xii + 393 pages. 228 figures. \$4.00.

There have been a number of textbooks published for the use of students and others dealing with arthropods and the transmission of disease during the past five or six years. The present volume differs from the most of these in that it introduces the subject with a concise description of parasitism and the morphology of the parts that have to do with transmission. This includes a statement as to the classes of parasites, the effect and origin of parasitism, and a tabulation with figures of the systematic position of animal parasites. There is a brief discussion of the internal anatomy, classification and metamorphosis of insects, with good illustrations of the various types. It is unfortunate that the author has introduced new names for the three types, as there are too many already. In practically all the insects that have to do with the transmission of disease, the mouth-parts are fitted for piercing or sucking. The derivation of this type from the simpler biting type is shown in detail, not only for those groups that are known to suck blood, but for all others. This is supplemented by a list of the orders arranged according to their type of mouth, together with a statement as to their type of metamorphosis. Such a treatment is to be commended, for the sucking type of mouth is distinctive in form and structure in each group where it occurs. The following sixteen chapters discuss fully: how insects cause and carry disease, direct and indirect infection, external and internal parasites; the life-history, habits and relation to disease of common household insects; the biting and sucking lice infesting domestic animals and man; the life-history, habits and relation to disease of the bedbug and cone-nose; the transmission of malaria, yellow fever and other diseases by mosquitoes, their habits, life-history and control; buffalo-gnats and their relation to pellagra; the house-fly and its relation to the transmission of intestinal diseases, together with measures for its control; the blood-sucking muscids and their relation to sleeping sickness and poliomyelitis; myiasis and the bot-flies; fleas and the transmission of bubonic plague; ticks and tick-borne diseases; mites as skin parasites; and the venom of bees, wasps, spiders and scorpions. There are also included analytical tables for the identification of adult mosquitoes, the families of the dipterous larvae producing myiasis, and the families and some of the genera of fleas.

The book can be recommended for its figures, most of them new, and for its carefully prepared, well-balanced subject-matter.

NOTE

Special courses in parasitology with emphasis upon field and experimental work are announced by Dr. George R. LaRue in the program of the Michigan University Biological Station at Douglas Lake for the summer of 1917.

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ON THE SPOROZOON PARASITES OF THE FISHES OF WOODS HOLE AND VICINITY

I. FURTHER OBSERVATIONS ON MYXOBOLUS MUSCULI FROM FUNDULUS

C. W. HAHN

My knowledge of several points relating to the life history, structure, and habits of *M. musculi* as described in a previous paper was incomplete. More recent studies have supplied interesting additions to and confirmation of previous observations. The new matter relates to the method of infection, the pathological effects, the mode of attack, the distribution of the disease within the species, and certain obscure stages of the life cycle.

DISTRIBUTION OF THE PARASITE IN NATURE

Hitherto my observations have been made upon fish that had been captive for one or more days. Since a very large proportion of them were found to be infected with both bacteria and Myxosporidia, there seemed to be good grounds for expecting fish at large to be infected in rather large numbers. But this does not seem to be the case. When *Fundulus* are carefully examined immediately after reaching the laboratory, the number of fish having lesions of any kind are surprisingly few. In one catch of one thousand fish, only eleven had pathological abnormalities. One of these eleven fish was infected with *Myxobolus musculi*. From another catch of one hundred and seventy-five fish, Myxosporidia were found only in three fish which had lesions in the integument, there being no other fish having injuries. In a third catch of sixty-five fish the integument had typical lesions containing the parasites in but two cases. All of these counts were made when the water was at a temperature lower than the maximum in the vicinity of Woods Hole. The earliest count made was in July of a remarkably cold season. The proportion having *Myxobolus* was 4.4 per cent, while those taken in the latter part of the month of August had as low as 0.1 per cent infected.

The temperature is no doubt an important factor. The water of the indoor aquaria is warmer than that of the ponds and bays where

the fish are commonly caught. This, in part, accounts for the larger percentage of infection in fish that have been confined a day or two. But there are two other factors. It has already been demonstrated (Hahn, 1913:193) that injuries to the integument encourage the entrance of the *Myxobolus*. An examination of the gills of a number of *Fundulus* has recently revealed the fact that *M. musculi* is far more common on the gills of fish that are apparently healthy than it is in the integument and muscle. Fish having injuries and confined in aquaria are therefore exposed to infection from the gills of a comparatively large number of previously infected fish. These facts explain the discrepancy in the distribution of the parasites as found in captive fish and in free fish.

EXPERIMENTAL TRANSMISSION OF THE *M. MUSCULI* AND THE
CONDITIONS FAVORABLE FOR RECOVERY

In order to confirm the results of previous experiments along this line, two experiments were undertaken. Twelve *Fundulus* were placed in one aquarium jar having a capacity of at least 5 gallons and supplied with running water. Six fish were put into a second jar for the purpose of a control. The six controls had incisions cut in the integument in exactly the same manner as the fish which were inoculated, but a sterile scalpel was used. Bits of tissue known to contain the myxospores of *M. musculi* were inserted into pockets made with a clean scalpel under the scales of the opercle and head of six of the twelve fish above mentioned. Similar bits of tissue were inserted into incisions made in the integument of the remaining six fish so as to be in contact with the body muscle.

By the second day after the operation, all of the eighteen fish were still active. The wounds of all had developed into open infected sores, due, no doubt, to the bacteria which enter from the water. But there was far greater activity in the wounds of the twelve fish which had received infected tissue. The adjacent integument was rough, swollen and the scales were loosened. In some the flesh was exposed for a distance around the incision and a thick layer of white flaky flesh was about ready to fall out of the wound. This condition is unmistakably due to the destructive work of the *Myxosporidia*. Those fish which had received infection in the head region had more or less inflammation in the vicinity of the lesion and in some cases it had spread under the jaw and to the opposite side of the head. In one case the roof and floor of the mouth were found later to be highly infected with *Myxobolus*. This fish and one of the controls died on the second day of the experiment. The latter had a bad wound which proved to have numerous myxospores. They probably entered the wound from the

water or found their way in some way from the gills of one of the controls. As stated before, recent observations have shown that *M. musculi* is rather common in the gills of fish that show no signs of disease. The same conditions apply to a second control which died on the third day. The other four controls recovered and lived throughout the period of observation.

By the sixth day five of the inoculated fish died from the effects of the *Myxobolus*. The parasite was found in the infected tissues in each case. Altogether, eight of these fish died, three escaped, and after twenty-three days the remaining fish had apparently recovered. The three that escaped were seriously afflicted when last seen.

This experiment was repeated with some slight modifications for the purpose of gaining more light upon the natural immunity of the host. Infected material was introduced under the integument of four *Fundulus* as follows: (1) Fragments of tissue containing myxospores were placed under the integument of the operculum; (2) the same material was introduced under the integument of another fish on the dorsal side just between the eyes; (3) infected material was pushed into slits cut into the integument around the mouth; (4) the infected tissue was introduced into the flesh on the left side of the body. These four fish were given plenty of food and fresh water. They had been confined for thirteen days so that it was safe to assume that there were no well developed infections at the beginning of the experiment. No controls were kept.

The locus of the infections all developed into conspicuous lesions. The fourth fish developed a large open sore, three-fourths of an inch in diameter, with white opaque flesh. It died on the sixth day. The muscle around the area over which the integument remained unbroken was rich in the trophic stages of the *Myxobolus*, including some propagative stages. In the tissue used to infect this fish there were few, if any trophoblasts of either propagative or multiplicative stages. Myxospores were very abundant and other propagative stages were probably present. It seems likely that the new host was infected by the latter. The rapid hypertrophy of the tissues is characteristic of the disease and tends to show that the fish has little or no defence when muscle tissue is attacked.

In Fish No. 1 the muscle of the back and sides was involved by some means, probably by the spread of the disease to the dorsal side of the operculum. Here again a typical lesion was developed and resulted fatally.

The fate of the other two fish was very different. After twenty-six days both were alive and their wounds were healing rapidly. At first, both these fishes appeared to have wounds sufficiently serious to cause their death. But the thin subdermal connective tissue over the skull

either does not conduct the parasites beyond the reach of immunizing agents as in the case of the body muscle, or saprophytic bacteria and their toxins have not the favorable conditions to poison the host that are provided when the infection occurs in body muscle. Inasmuch as there is ample evidence that *M. musculi* does attack epidermis and connective tissue, one must conclude that in this case either the defense of the fish was sufficient to destroy the parasite before it spread to the body muscle or that the parasite passed through its trophic stages and had become non-virulent. In the fish which received infection through the muscles of the lower jaw, there was nothing to limit the spread of the virulent stages into muscles where it would be fatal, such as the eye muscles. One is therefore inclined to the view that the parasites pass into a comparatively inactive condition. This would require a very simple explanation, namely, that the trophic stages develop simultaneously into sporogenic stages. Such was doubtless the case with most of the parasites in the primary host. In the latter the disease never at any time assumed very injurious conditions. Yet I have observed cases of infection in the head region which resulted fatally. This particular fish lived for over a month after the disease was first observed on the middle of the opercle. It did not spread beyond the border of the opercle, and when last observed at the end of the season the wasted tissues were rapidly regenerating. At the start, myxospore and sporoblast stages alone were encountered in large numbers. All of the parasites seem to have developed into sporoblasts and eventually myxospores so that the host was safe for the season unless the spores germinated again. In the two fish mentioned above, the transfer of the myxospores to another host apparently supplied the necessary stimulus, or there were still a number of trophoblasts of the propagative cycle.

The conditions of the recovery in these three cases were chiefly the location of the primary infection. Had the fish not been well fed, they would doubtless have died, as have many others having infected jaws, eyes, opercles, etc. But food alone will not explain their recovery, because I had here two and have had at other times many other fish with infections in the body muscle which nearly always kill the fish.

Recovery in the barbel when afflicted with abscesses caused by *M. pfeifferi*, is possible when there is no external lesion or when no vital organ is involved. Usually these are the conditions when the body muscle alone is infected. According to de Drouin de Bouville (1908), phagocytosis then prevents the fatal accumulation of atrophied tissue. As has been already observed, the conditions are just the contrary in the *Fundulus*. When *M. musculi* invades the body muscle it is rarely checked and when the attack is superficial as in the head region, the chances of recovery are good. In this conclusion I have assumed that

the myxospores of *M. musculi* are not capable of germinating in the tissues where they have matured. Mercier (1906) has established this as a frequent method of multiplication in *M. pfeifferi* of the barbel. Altogether, the evidence that the myxospores of *M. musculi* may germinate in the original host is negative. The fact that numerous myxospores were observed unaccompanied by other stages for such a long period in the case above mentioned is, in itself, a sufficient proof that, in this case at least, the necessary stimulus for germination of the myxospore was lacking.

In regard to the propagation of *M. musculi* from fish to fish, it may eventually prove that the myxospores may enter the tissues both through lesions as is indicated by the above experiments, and through the gills and through the digestive tube. Since it has been shown that *M. pfeifferi* is taken into the barbel with its food, the latter mode of infection for *M. musculi* seems the more probable, especially when it is recalled that the relations of both parasites to their host are so very similar. The attack upon the muscle fibers is almost identical in the two species.

The myxospores of *M. inequalis* which causes the disease known as carp pox, are also transmitted to new hosts by means of the food (Wierzejsky, 1898).

Contrary to my expectation, there is absolutely no evidence that *Fundulus* ever suffers from an internal infection by *M. musculi*, unless it be about the mouth and gill region.

In the summer of 1915 I again inoculated fish with Myxosporidia. In these experiments the ultimate object was to discover if the species of *Myxobolus* hitherto commonly encountered in *Fundulus heteroclitus* would grow and produce the same typical pathological conditions in *F. majalis* and *F. diaphanus*, and to see if the parasite could be recovered in the same host in one of its characteristic stages.

A *Fundulus heroclitus* which proved by examination of stained tissues to have typical large schizonts in considerable numbers was first secured. From two typical *Myxobolus* lesions in the lateral region of the body, bits of flesh about 1 by 3 mm. in size were removed by means of sharp sterilized forceps. The subjects were confined in clean aquaria, with running sea water for *F. majalis* and *F. heteroclitus*, and fresh water for *F. diaphanus*. They were fed regularly each day. Inasmuch as it has been shown that lesions free from Myxosporidia in fish which are well cared for rapidly recover, no controls were provided. This was partly due to the fact that one cannot be sure that the water is free from Myxosporidia, since the gills of many *Fundulus* may be infected and presumably disseminate the germ.

The results of operations upon thirteen fish are summarized in the following table:

TABLE 1

1	2	3	4	5	6	7	8	9
Species	Catalogue Number	Length in inches	Time of Inoculation	Time of Examination	Period of Growth	Dead or Killed	Condition of Wound (gross exam.)	Kind of Organism (based on examination of stained tissue)
<i>Fundulus majalis</i>	1097.1	5.5	8/24/15 12 a. m.	8/26/15 7 a. m.	19 hrs.	Died; water supply failed	Large sore on site of incision	Muscle degenerate; schizonts many in muscle
<i>Fundulus majalis</i>	1097.3	4.5	8/24/15 12 a. m.	8/26/15 7 a. m.	19 hrs.	Died; water supply failed	Large lesion, advanced	Many schizonts; muscle degenerate*
<i>Fundulus majalis</i>	1097.5	5.5	8/24/15 12 a. m.	8/26/15 7 a. m.	19 hrs.	Died; water supply failed	Moderate sized lesion	Schizonts large but few
<i>Fundulus majalis</i>	1097.7	3.8	8/24/15 12 a. m.	8/26/15 7 a. m.	19 hrs.	Died; water supply failed	Large lesion, advanced	Muscle badly degenerate; not many schizonts, probably too degenerate
<i>Fundulus majalis</i>	1097.13	3	8/24/15 12 a. m.	9/ 6/15	13 days	Killed	Lesions $\frac{1}{4} \times \frac{1}{4}$ ", open but shallow, white	<i>Slide Lost</i>

All except one produced serious lesions.								
<i>Fundulus aphanus</i>	1097.6	4.8	8/24/15 12 a. m.	8/26/15 7 a. m.	19 hrs.	Died	Moderate lesion	Schizonts in small numbers
<i>Fundulus aphanus</i>	1097.8	4	8/24/15 12 a. m.	8/26/15 7 a. m.	19 hrs.	Died	Large lesion; open wound; purulent flesh	Many schizonts and some trophoblasts
<i>Fundulus aphanus</i>	1097.10	2.8	8/24/15 12 a. m.	8/26/15 7 a. m.	19 hrs.	Died from the infection	Large, with inflammation extending to pectoral fin	A few schizonts
<i>Fundulus aphanus</i>	1097.11	2.8	8/24/15 12 a. m.	8/26/15 7 a. m.	19 hrs.	Died from the infection	Moderately developed lesion	Schizonts rare
<i>Fundulus aphanus</i>	1097.12	3.3	8/24/15 12 a. m.	8/27/15	3 days	Died from the infection	Large area with inflammation extending over ribs	Muscle degenerate; large schizonts not numerous

* Schizonts sporulating.

Extensive lesions developed in all. Schizonts present in all but rather rare.

TABLE 2

1	2	3	4	5	6	7	8	9
Kind of Fish	Catalogue Number	Length in Inches	Date of Inoculation	Date of Examination	Period of Growth	Dead or Killed	Condition of Wound	Stage of Parasite
<i>Fundulus heteroclitus</i>	1103.1	3	8/31/15 12 a. m.	9/1/15 10 a. m.	22 hrs.	Died, probably shock	Lesion almost unchanged....	Trophoblasts present but very rare
<i>Fundulus heteroclitus</i>	1103.3	4	8/31/15 12 a. m.	9/1/15 3 p. m.	27 hrs.	Died, probably from head infection	Gills diseased; head and eye infected; lesion unchanged	Mycocysts in gills; large trophoblasts in few; muscle degenerate
<i>Fundulus heteroclitus</i>	1103.6	3.5	8/31/15 12 a. m.	9/2/15 9 a. m.	45 hrs.	Died	Badly infected about head; eye and opercles protruding; lesion not changed	Trophoblast in gills; large trophoblasts, many, in muscle
<i>Fundulus majalis</i>	1103.7	4.5	8/31/15 12 a. m.	9/6/15	7 days	Alive	Wound almost invisible; no evidence of disease	Schizonts few; many small trophoblasts
<i>Fundulus majalis</i>	1103.8	3.5	8/31/15 12 a. m.	9/6/15	7 days	Alive	Wound still open; no evidence of infection	Many trophoblasts
<i>Cyprinodon variegatus</i>	1103.2	1.5	8/31/15 12 a. m.	9/1/15 10 a. m.	22 hrs.	Died	Lesion not developed.....	Young trophoblasts in muscle many; muscle degenerate
<i>Cyprinodon variegatus</i>	1103.4	1.8	8/31/15 12 a. m.	9/1/15 3 p. m.	27 hrs.	Died	Slight infection about head; lesion but little developed	Muscle full of trophoblasts; gill has sporoblast stages
<i>Cyprinodon variegatus</i>	1103.5	3.5	8/31/15 12 a. m.	9/2/15	45 hrs.	Died	Inflammation on head serous; swollen eye and opercle; incision not changed much	Many large schizonts and trophoblasts; Myxocysts in gills

The right-hand column of the above table indicates the kind and number of *Myxobolus* in the hypertrophied tissues, especially muscle, of the operated fish. In twelve out of thirteen fish the *Myxobolus* was recovered after being introduced. In all cases it had multiplied and was growing in a perfectly normal way. There is no evidence that the change of host has modified the usual course of the life cycle.

Considering the last two columns together, one may conclude that the parasite encountered a favorable medium for growth in all three of the species concerned. In *F. diaphanus* there is a marked difference in the abundance of the *Myxobolus* as compared with either *F. majalis* or *F. heteroclitus*. In two cases of *F. majalis*, one is justified in assuming that there were large numbers of parasites, though they were not actually seen, because in one case the fish died of the disease and the slide preparation of its tissues was in some way lost; in the other case the extremely degenerate condition of the tissue justifies one in the expectation that no parasites will be found. Had the slide included muscle near the edge of the lesion, it is certain, on the basis of previous observations, that a large number of parasites would have been found.

One may conclude so far as this experiment goes that *F. diaphanus* is less favorable to the growth and multiplication of the *Myxobolus*. By reference to Columns 6 and 7, it is clear that, notwithstanding the smaller number of parasites, the disease is equally if not more destructive, having produced extensive necrotic sores and killed all specimens of *F. diaphanus* in three days. The unfortunate failure of the sea water at the end of nineteen hours prevented an interesting comparison of the endurance of the three species with reference to this parasite.

These observations prove beyond doubt that there is a succession of multiplicative cycles, and that large trophoblasts do not pass directly into the propagative condition. The propagative stages are distinctive and easily recognized both by their habit and staining qualities. It is now certain that some considerable multiplication in the multiplicative individuals involving several cycles must intervene before the propagative trophoblasts are produced.

The objection may be made that the culture utilized in the above-mentioned experiments was not pure, since one fish known as 1097.9 proved to be afflicted with both *Chloromyxum funduli* and *M. muscoli*. It is necessary to admit that one could not with precision distinguish the trophoblasts of the *Chloromyxum* from those of the *Myxobolus* unless conditions happened to be very favorable. This is not the case, however, if either of these parasites are in the propagative cycle. In this case all the stages are distinctive for the two genera. There are besides this two very good reasons for believing that the fish from which these primary cultures were taken did not harbor *Chloromyxum* to the exclusion of *Myxobolus*: (1) The Fish 1097.9 is the second case

of *Chloromyxum funduli* which I have observed in the tissues of many hundred infected Fundulus; (2) no recognizable stages of *C. funduli* could be found in the material available in any of the other twelve fish mentioned above. One would hardly expect this particular combination of circumstances which would provide only one example of a parasite in the propagative cycle when they usually advance simultaneously from stage to stage, and at the same time that the initial infection be of rare occurrence, one which is encountered about one time in two hundred.

The inoculation experiments which follow are of a similar character to the above, and give support to and throw additional light upon some of the conclusions mentioned above. The purpose, however, was to aid in solving two questions which arise from the following circumstances. I have observed slight differences in the size of the myxospores from the gill and from the flesh of the Fundulus. In the gill I have encountered a range of variability in length from 13.4 to 12 μ ,

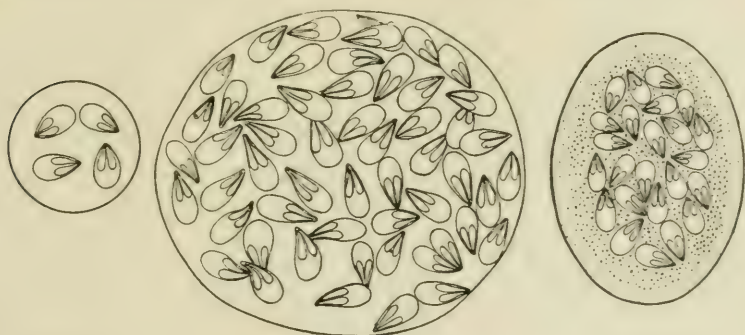


Figure 1

Figure 2

Figure 3

Fig. 1.—Cyst from gill filaments of Fundulus containing four myxospores of *M. musculi*. The cytoplasm around the myxospores is unstained. In this particular gill there were a number of these cysts.

Fig. 2.—Cyst from gill filaments of Fundulus containing a small number of myxospores of *M. musculi*. A conspicuous granular cyst plasma with definite outer wall characterizes this common type of encystment in the gill.

Fig. 3.—Cyst from filaments of Fundulus containing a large number of myxospores of *M. musculi* which have been assembled without any evidence of surrounding cyst plasma. There is, however, a definite limiting membrane. 68 by 67 μ .

and in width from 10.4 to 6 μ . For those seen in the flesh we have recorded elsewhere an average length for apparently mature myxospores of 14.3 μ and an average thickness of 6.7 μ . For obviously immature myxospores the dimensions average about 12 by 7.5 μ . The size difference is therefore rendered invalid as an evidence of difference by the element of age. Another possible specific difference is suggested by the occurrence of myxospores both singly and in sporocysts of different sizes (Figs. 1, 2, and 3) in the gills, whereas in the flesh they

are usually isolated in our smear preparations. This difference can scarcely be due to the process of making smear preparations, because one should at least find the myxospores clustered if not occasionally in pseudocysts. It is very probable in view of what follows that the myxospores are either mechanically aggregated in the gills or if normally so related, they are mechanically distributed by the action of muscular contraction.

In order to finally settle this question of identity it was planned to introduce some of the myxospores of the gill, and if it so happened, some of their related trophic stages, into the body muscle. If the species were not identical, one would expect a marked difference in the pathological conditions and general habit of the parasite, if indeed it would grow at all. Some entire gill filaments of *F. heteroclitus*, 1098, which contained the myxospores of a *Myxobolus* in large clusters, singly and in sporocytes having four myxospores in each (Fig. 1), and large multiplicative or possibly propagative trophoblasts, were introduced under the integument of a *F. heteroclitus* 6.5 inches long. In four days the infected fish was dying. The mouth was gaping and there was an acute inflammation around the mouth and head. A large lesion had developed around the incision and the adjacent flesh under the unbroken swollen integument was a purulent mass. It was a typical myxosporidian wound. The muscle fibers of the fish were abundantly infected with numerous small multiplicative trophoblasts, many large trophoblasts and also large masses of multinucleated sporoblasts.

Unfortunately the water with which these fish were supplied was exposed to contamination by other infected fish. The head infection was doubtless due to direct contamination by handling or to the infected water. But I believe the flesh to have received its deep-seated and profound infection from the fragment of gill which was introduced.

The contaminated water made it necessary to repeat the experiment. As a number of *Cyprimodon variegatus* were available it was planned to test the possibility that *M. lintoni* and *M. musculi* are one and the same species (Hahn, 1913: 206). The gill filaments containing one or more large pseudocysts composed of apparently mature myxospores of the genus *Myxobolus* were removed from *F. majalis*. After carefully isolating a single filament it was introduced under the integument overlying the body muscle. The details of the experiment with summary of the observations will be found in Table 2.

It should first be noted that Fish 1103.1 died in less than a day, and thereupon in Column 8 the visible injury is found to be slight. The same condition prevails in 1103.3, but in 1103.2, 1103.4, 1103.7, and 1103.8 no reason can be given for the non-development of a typical lesion.

If one considers Column 8 it is impossible to deny that in some cases, at least, typical lesions do develop; but the evidence is not conclusive. The regular occurrence of one or more stages of the parasite in the flesh as indicated by Column 9 certainly forbids the conclusion that the myxobolus of the gill will not grow in the flesh. Allowing for the fact that one does not always happen to include in a smear preparation Myxosporidia when present, it may be assumed that all the tissues reported in Column 9 contain multiplicative trophoblasts. No propagative stages were encountered. In those fish that lived forty-five hours and seven days were found large trophoblasts and stages which I have considered practically mature, i. e., schizonts. This fact harmonizes with the assumption that the transplanted myxospores have given rise to the new infection.

When compared with Columns 8 and 9 of Table 1, Columns 8 and 9 of Table 2 are not strikingly different, especially if one takes into consideration the period of development (twenty-two to forty-five hours), and a possibly longer time required for a myxospore to germinate. One must also consider the relative numbers of individual parasites represented in a bit of flesh containing hundreds of individuals and a bit of gill filament with only one or two pseudocysts like that in Figure 3. Obviously far more significance must be attributed to the presence of parasites at all, as indicated in Column 9, than is at first apparent. Considering the fragile nature and the relative size of myxospores which vary at different stages of development, and the difference in the nature of pseudocysts which may be either mechanical or due to too limited observations, I feel justified in taking the view that there is but one species of *Myxobolus* in *Fundulus*, and that it can be transplanted both by myxospores and trophic stages.

The case of the identity of *M. musculi* and *M. lintoni* is more perplexing. Since *M. musculi* grew readily (see Table 2) in *F. diaphanus* from fresh water, it might be supposed that it would grow more or less in the flesh of *C. variegatus*. If, on the other hand, the growth in *C. variegatus* had produced a typical tumor and the large type of myxospore had been recovered (Hahn, 1913:206) we might find in the above observations evidence of the identity of the two species. It should be recalled that the *M. lintoni*, described by Linton (1891) and Hahn (1913), produced in all cases a very characteristic dermal tumor, which caused the death of the fish, according to Hahn, in a period of from two to three days. Such tumors are never encountered in the *Fundulus*, and nothing suggesting them was produced in the *Cyprinodon* of this experiment. On the other hand, *M. musculi* produces a typical ulcer in every way comparable to that in *Fundulus*.

It is worthy of note that, though the number of cases is small, there was an apparent difference between the number of parasites found in

F. heteroclitus and in either *F. majalis* or *C. variegatus*. This, together with the slight difference in the degree of development of the lesion, indicates that the parasite grows more readily in the muscle of *F. majalis* and *C. variegatus* than in *F. heteroclitus*. The number and maturity of the myxospores introduced must be taken into account. The conclusion is therefore not positive.

The above experiment, of which Table 2 is a summary, furnishes a minor contribution to the life-history of *M. musculi*. It would appear that if a pure myxospore culture were used in the inoculations, and if after seven days large schizonts are found in the second host, that not more than seven days is required for the parasite to pass from sporoplasm to schizont. Reference to Columns 6 and 9 of the table shows that such was the case in Fishes 1103.7 and 1103.8. But Fish 1103.5 had many large schizonts which must have developed in a forty-five-hour period. At least one cycle may therefore be completed in forty-five hours, and probably less, since the number of individual parasites in the twenty-two-hour cultures was far greater (1103.2) than the number of myxospores introduced. This conclusion is not absolutely certain inasmuch as a gill filament containing a pseudocyst might also contain other stages unseen, but it is very improbable if the myxospores are ripe. If trophoblasts were present, they were not numerous and the time relations above recorded would then apply to the period of a cycle starting with a multiplicative spore rather than a myxospore.

The ease with which one can introduce either multiplicative spores and trophoblasts or myxospores and probably propagative trophoblasts into the tissues of a healthy fish provides a very plausible explanation of the way by which fish whose integument has been broken may pick up the *Myxobolus* from the water. Thus, though commonly in the gill and head region where it is comparatively harmless, it comes to react on the body muscle where it is oftentimes fatal. Rough handling and close confinement in aquaria tend to provide the ideal conditions for the infection of the muscle.

A final solution of a question which confronted the writer during the first stages of his investigations of *M. musculi* (Hahn 1913: 199), namely the possible causative relation of certain bacilli to the pathological changes in *F. musculi*, is found in the inoculation experiments. When one can produce at will the typical condition by the use of *Myxobolus* myxospores but fails to get it by laceration, one may conclude that the bacteria are purely secondary. Moreover, numerous preparations show that the vanguard of the infection is always a tissue comparatively free from bacteria. I am not prepared to say that bacteria do not poison and kill the host as secondary agents. They are probably saprophytic and the primary *Myxosporidian* parasite prepares the way for them.

SUMMARY OF RESULTS OBTAINED IN INOCULATION EXPERIMENTS

1. *M. musculi* is communicable in all stages of its life-history.
2. Many multiplicative cycles are repeated before *M. musculi* passes into its propagative cycle.
3. The Myxobolus which is very common in the gills, where it is seldom destructive, is identical with that which occurs in the flesh.
4. Infection of lesions in the integument takes place upon the entrance of any stage of *M. musculi* from contaminated water. The water is presumably contaminated from the gills.
5. Transplanted *M. musculi* may continue for some time in the same cycle in the new host. Or they may pass into the next cycle soon after the transfer.
6. Myxospores germinate when transplanted to another fish and produce schizonts in considerable less than one day.
7. The multiplicative cycle requires less than one day and probably takes place many times in this period.
8. The propagative cycle may be reached in 48 hours.
9. Recovery from infection with *M. musculi* is possible if the body muscle is not involved and if the fish are fed and supplied with oxygen. Eye muscles and possibly other parts of the head are also vital.
10. Recovery is probably possible if the infection occurs when the parasite is in or near the end of the propagative cycle even when the body muscle is involved.
11. Progress of the disease is slow in the integument.
12. The parasite almost invariably migrates from hypertrophied tissues.
13. Passage from stage to stage is approximately simultaneous.
14. *Fundulus majalis*, *heteroclitus*, and *diaphanus* and *Cyprinodon variegatus* are culture media for *M. musculi*. *C. variegatus* is a little less favorable for its growth but is perhaps less immune to the toxic products evolved in this particular kind of a lesion.
15. The Myxobolus from the gill of *Fundulus* is identical to that which is common in the flesh.
16. There is no valid reason as yet to consider *M. musculi* and *M. lintoni* of the *Cyprinodon variegatus* as one and the same species. On the other hand the bulk of the evidence favors the opposite view.
17. The associated bacteria are either purely saprophytic or secondary parasites which gain entrance from the water and find the natural resistance of the tissues lowered by the Myxobolus. The latter invade the normal tissue and leave the atrophied tissues to the bacteria.

Observations upon the multiplicative stages of *M. musculi* will follow in an early number of THE JOURNAL OF PARASITOLOGY.

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NOTES ON THE CERCARIAE OF THE BITTER ROOT VALLEY, MONTANA *

ERNEST CARROLL FAUST

Before the work of Cort (1915) only isolated accounts of North American Cercariae had been published. Most of these lacked the detail necessary to distinguish species of the same groups which bear a superficial resemblance to one another. The general idea which Charles Sedgwick Minot voiced is just as true for larval trematodes as for any other larvae, when he said "It is not true that embryos are alike; on the contrary, they show class, ordinal, and generic differences from one another." This has been the case with the trematode forms that have come under the observation and consideration of the writer. Some details of difference are visible from a study of the living animals. Other points of differentiation require a specific, altho not unusual technic.

During a two-years residence at Missoula, Montana, the writer became acquainted with the fauna of the Bitter Root Valley. One of the striking features of its fauna is the small number of species, altho the number of individuals of each species is large. In contrast with this fact is the large number of parasites found in the aquatic fauna of the region. Thru the courtesy of Mr. Bryant Walker of Detroit, Michigan, who has identified shells from some fifteen collections, the writer has ascertained that the gasteropod fauna of the vicinity consists of three species, *Lymnaea proxima* Lea, *Physa gyrina* Say, and *Planorbis trivolis* Say. Thirteen species of cercariae have been secured from these snails, species embracing three groups of Digenea. In addition, a fourteenth species, a *Diplostomulum*, has been found in the squawfish, *Ptychocheilus oregonensis* Richardson. Of these fourteen, two are Monostomata, two are Holostomata, and the remaining ten belong to the Distomata. The writer wishes to take this opportunity to thank Professor Henry B. Ward for many valuable suggestions, and to express his gratitude to Mr. Norbert Sager for faithful collection of material during the summer and fall of 1916.

Collections were made along the Bitter Root River for a stretch of some fifty miles from Hamilton to the confluence of the Bitter Root and Missoula Rivers. One collection came from Rattlesnake Creek, Missoula. From this latter collection, made in November, 1916, were

* Contributions from the Zoological Laboratory of the University of Illinois under the Direction of Henry B. Ward, No. 80.

secured one species common to several localities along the Bitter Root stream, and one new species not found in the Bitter Root Valley.

All of the species examined have proved to be new. Preliminary studies have been made on living specimens to note the general behavior of the organism and to work out the details of the excretory system. Other organs and tissues have come out more advantageously from preserved and stained mounts. Extremely satisfactory results have been obtained from material fixed in Gilson's fluid and stained with Delafield's and Ehrlich's acid hematoxylin.

MONOSTOMATA

Three monostome cercariae previously described for North America are: *Cercaria hyalo-cauda* Haldeman 1842; *C. (Glenocercaria) lucania* (Leidy) 1877; and *C. urbanensis* Cort 1914. Two new species are contributed from the Bitter Root Valley, *Cercaria pellucida* and *C. konadensis*.

CERCARIA PELLUCIDA *nov. spec.*

[Figure 1]

A light infection of *Cercaria pellucida* was found in the liver tissue of *Physa gyrina* Say, collected near Fort Missoula. A much heavier infection of the same species was secured from *Lymnaea proxima* Lea at Corvallis, Montana. The cercariae have an average length of 0.4 to 0.7 mm., and a width of 0.18 to 0.2 mm. The tail measures 0.5 mm. in length by 0.07 mm. at the base. The anterior end of the body tends to be bluntly fusiform, while the posterior part is elongate ovoid, with two symmetrically placed projections where the pair of locomotor pockets extend posteriad and ventrad. The pigmentation is prominent, centering around the two paired, lateral eye-spots, and a third which is single and median. The pigmentation proceeds posteriad along six lines, two lateral, two dorsal and two ventral. A single sucker at the anterior extremity is aided in locomotion by the paired lateral pockets, situated at the posterior extremity of the body somewhat lateral to the junction of trunk and tail.

The animal has a typical "measuring worm" movement, produced by the action of the oral sucker and the locomotor pockets, accompanied by alternate contraction of longitudinal and transverse muscles. The "turbine movement" of the tail also pushes the animal forward thru the medium. The body of the cercaria is smooth, covered with a heavy integument, and the inside of the pharynx has a spinose lining.

The cercaria is produced in a redia with a tough integument and well developed musculature. The redia measures 2.2 by 0.5 mm., and has a long single gut-pouch 2.0 by 0.3 mm. The gut is filled with reddish orange pigment caused by the digestive action of the organ on

the liver tissue of the host. The pharynx is strongly muscular and possesses a four-lobed evertible prepharynx with a spinose covering. The rhythmic driving of this organ against any object with which it comes in contact constitutes the most characteristic movement of the redia. The germinal epithelium is situated in the posterior part of the redia.

The cercaria usually remains in the redia until it is mature and ready to seek a new host. A slight pressure on the redia causes it to burst at the anterior end, and the rent allows the mature cercariae to escape. They do not remain long in the surrounding medium (water or liver tissue) before encystment. This process is extremely rapid. A mucoïd is first poured out around the wriggling worm and within this a granular area which acts as a liquid cushion for the cercaria. During the process of encystment the cercaria has coiled upon itself so that the resulting cyst is spherical. Encystment is so rapid that the tail is not dropped until the completion of the cyst.

Cercaria pellucida is characterized superficially by no special feature that would differentiate it from any of the larger species of monostome cercariae with three pigment eye-spots. Internal characters, however, readily distinguish this form from previously described species. The locomotor pockets are smooth internally, differentiating the species from *C. imbricata* Looss (Looss, 1896) and *C. ephemera* Nitzsch (Ssinitzin, 1905). The tail has no central gland cells, so that the common median excretory tube is surrounded by the ordinary parenchyma cells. The excretory system of the trunk consists of a complete circuit with the anterior extremity just posterior to the median pigment eye, and the posterior limit of the system in the bladder. When contracted, the bladder is superficially triangular with the excretory pore caudad. The system is filled with many large granules of a high refractive index. They are probably of a derived protein nature and disappear on application of strong acids and alkalies. However, after treatment with a non-acid fixing agent (mercuric chlorid), they are acid-resistant.

The eye-spots consist of a terminal ganglion cell for each spot, surrounded by a pigment cup. The eyes open dorso-laterad. They have a direct connection with the brain.

Most specific of all the systems are the genital organs of *Cercaria pellucida*. The germaria (ovary and two testes) are situated in the posterior reaches of the trunk just anterior to the excretory vesicle. The ovary is median; the minute testes are lateral to the ovary. No Laurer's canal has been found. The vitellaria consist of five pairs of glands within the confines of the excretory circuit and three pairs situated more laterad. They cover a considerable area of the ventral surface. The glands are filled with fine granules closely associated.

The ducts from the glands traverse the region on each side intermediate between the inner and outer series, and meet one another in the region of the ovary, emptying thru a common duct into the ootype. From this region there proceeds forward along a median line the long slender uterus, which enlarges just behind the median eye to form the vagina. To the left of the uterus is the common vas deferens which has resulted from the junction of the vasa efferentia just anterior to the ovary. It runs parallel to the uterus, and at its anterior end expands into a cirrus pouch.

The body is crowded with cystogenous gland cells filled with rhabditiform granules. Crowded in between these cells are the parenchyma cells and connective tissue. The cystogenous cells with their contents give to the worm a milky translucent appearance.

The digestive ceca are given off from the esophagus just behind the plane of the paired eye-spots and extend to the posterior extremity of the body. Their lumina are filled with a jelly in which are granular inclusions.

CERCARIA KONADENSIS nov. spec.

[Figure 2]

From the same individuals of *Lymnaea proxima* Lea at Corvallis, Montana, from which *Cercaria pellucida* was obtained, there were found in lesser numbers specimens of another new species of monostome cercariae for which I propose the name *Cercaria konadensis*, the specific designation of which is derived from the Indian term for Bitter Root.

This species is very unlike *Cercaria pellucida*. It is considerably smaller, having a length of 0.4 to 0.46, and a width of 0.1 to 0.16 mm. The tail is equally long and has a transverse diameter of 0.03 to 0.04 mm. at the base. The anterior end is lanceolate, as is also the extremity of the tail. Superficially, the most striking feature of this cercaria is the lack of the median pigment eye, accompanied by a lesser amount of pigmentation in the posterior half of the body. This stands in contrast with the trioculate monostome cercariae where the pigmentation is more extensive. In this respect *Cercaria konadensis* bears similarity to *C. urbanesis* Cort (Cort, 1915), which has only two true eyes and a median condensation of pigment, and contrasts with *C. ephemera* Nitzsch (Ssinitzin, 1905; Lebour, 1905), and *C. imbricata* Looss (Looss, 1896).

The germinal epithelium of the redia in which the cercaria develops is modified into a central rachis which is proliferated from the posterior part of the body. This redia is much smaller than that of the trioculate cercariae, with a length measurement of 1.7 mm., and a diameter in cross section of 0.35 mm. The pharynx is comparably

smaller and the gut extends only about three-fifths the way to the posterior extremity. It is about 1.0 mm. long and about 0.1 mm. in diameter. There is no oral armature. The walls of the redia are muscular and fairly well covered with integument.

Superficially, *Cercaria konadensis* cannot be distinguished from *C. urbanensis* Cort, altho the writer believes it is on the average more attenuate than that species. On the other hand, there are internal characters that readily allow a differentiation.

The posterior locomotor pockets are lined on their inner faces with a group of ten to twelve gland cells which probably pour out a secretion for attachment of these organs to the surface of the contact objects. There exist six paired groups of glands in the tail, lying just laterad to the caudal excretory tube. Each group constitutes a rachis, with the broadened end of mature cells directed toward the trunk, and the acute end of proliferating cells directed distad. This paired series of twelve groups of glands in the tail constitutes the readiest mark of distinction for the species. It separates it from *C. pellucida*, on the one hand, and on the other from *C. urbanensis* Cort, in which the writer has found six pairs of glands in the tail, each gland composed of a single polygonal cell. The exact number of these cells is not stated by Cort (1915), altho he mentions their presence.

The excretory system is not dissimilar on the whole to that of other monostome species. The bladder is small, 14 to 15 μ thru the longitudinal axis of the larva and 16 to 17 μ in breadth. The excretory tubes empty into the bladder from the extreme antero-lateral angles. The general aspect of the vesicle is a strongly compressed spheroid. The excretory pore is dorsal.

The genital fundamentals are hardly as clearly outlined in *Cercaria konadensis* as they are in *C. pellucida*. The ovary is just anterior to the excretory bladder, is pyriform in shape and lies dorsad to the ootype. It has a distinct Laurer's canal. The testes are slightly postero-lateral to the ovary. The cells of these glands are poorly defined, altho the efferent duct of each is indicated by a double row of cells. These ducts lead into a common efferent duct anterior to the ovary, and this runs forward to the right of the uterus, ending in a bulbous cirrus pouch a slight distance behind the vagina. As is usual for monostome cercariae, the yolk glands consist of a paired inner series of five glands and a paired outer series of three glands. They are very diffuse, dendritic, and are readily traced to the common lateral ducts which proceed posteriad, and, finally, turning mesad in the plane of the ovary, empty thru a common vitelline duct into the ootype. The uterus reaches from the region of the ootype to the plane of the anterior vitelline glands, where it enlarges into the vagina. This loca-

tion of the genital atrium is considerably behind the two eye-spots, so that this feature distinguishes it from that of *C. pellucida*.

The eye-spots are two in number situated superficially to the right and left of the roots of the posterior dorsal nerve trunks from which they receive innervation. Pigmentation is centered around the brain and its immediate nerve trunks.

Cystogenous glands fill the greater portion of the connective tissue complex. Encystment is rapid. This species is precocious in that it encysts frequently within the tissues of the snail.

HOLOSTOMATA

Few Holostomes, either in larval or adult condition, have been described for North America. *Diplostomum cuticula*, *D. grande*, *D. volvens*, and *Tetracotyle typica*, all Old World forms, have been reported for North America, and Stafford (1904) has described a new species, *Diplostomum parvulum*. The descriptions of these forms are not detailed, and it is doubtful if they are sufficient for exact determination of the species. An undescribed hemistome larva occurring in the vicinity of Urbana, Illinois, has features common to all of these, and especially characteristic for *Diplostomum cuticula*; yet it is undoubtedly a new species, as determined by internal structure. An isolated case of a holostome larva yet unnamed has been recorded by Rettger (1897).

The writer has found two holostomate larvae in the Bitter Root fauna, one a representative of the Hemistomes, and one a representative of the Holostomes.

CERCARIA PTYCHOCHEILUS nov. spec.

[Figure 3]

This species was found in very large numbers (several thousand) in the agamic stage in the mesentery of *Ptychocheilus oregonensis* Richardson, caught at Stevensville and Carlton, Montana, in April, 1915. As a hemistome larva encysted in a vertebrate, it is technically a *Diplostomulum*. The stage in the mollusc has not been secured. The larvae were included in large vesicular cysts of a translucent consistency. The cyst was attached to the mesentery by a disc. Upon transfer to normal saline or Ringer's solution the end of the cyst was split and the larva emerged.

Cercaria ptychocheilus is conspicuous because of its abbreviated posterior portion and its elongate patelliform anterior region. The body averages from 0.48 to 0.63 mm. in length by 0.17 to 0.37 mm. in width. There is a medium-sized pharynx. The ceca extend to the

acetabular region. The oral sucker is small and the acetabulum somewhat larger.

The excretory system consists of a bellows-shaped bladder into which leads a single median excretory trunk. In the mid-acetabular region it divides into three trunks, one branch proceeding forward and one each directed laterad. The lateral trunks form anastomoses both anteriorly and posteriorly. The median trunk continues its course unbranched until it approaches the region of the forking of the digestive tract, where it branches. The branches bend laterad right and left, and join the anterior anastomoses of the lateral trunks. Thus the system is bisymmetrical and constitutes a double circuit for the conduction of the excretory products. All of the tubules are filled with granules.

The genital system is typically holostomate, with the genital pore posterior. A muscular organ anterior to the acetabulum represents the original genital pore, which has lost its connection with the genitalia. The ovary is a club-shaped organ lying transversely posterior to the acetabulum and continuous mesad with the oviduct on the left side. The vitellaria are diffuse, ventro-lateral. No uterus is present in the larva. Two testes are situated on the right side, ventral and posterior to the ovary. No vasa efferentia or vas deferens is present. The genital pouch lies ventral to the excretory bladder. It is muscular and has paired groups of glands emptying into it.

CERCARIA FLABELLIFORMIS *nov. spec.*

[Figure 4]

This holostome larva possesses a right and left sucking disc in addition to the oral and ventral suckers; in consequence it belongs to the group designated as tetracotyle larvae. The species was found in the livers of a large percentage of *Physa gyrina* Say from three collections in the vicinity of Corvallis, Montana, in October, 1916. Some of the parasites were encysted, others were free in the liver tissue, and still others were in the redia. No mature cercariae were found in the rediae. The animal is broadly spatulate from ventral aspect. The length of the mature cercaria is 0.48 to 0.56 mm., and the width, 0.44 mm. The redia measures about 0.5 mm. in length and 0.16 mm. in width. The rhabdocoel gut is short. A pharynx is present. The birth-pore is situated on the ventral side, slightly lateral. Within the redia the germ balls are developed from the germinal epithelium localized at the posterior end. These balls develop into other rediae or tetracotyle larvae, both within the same redia.

Characteristic of the younger *Cercaria flabelliformis* is the tetracotyle suckorial apparatus, consisting of the oral and ventral suckers

and two lateral sucking disks. Behind the acetabulum are two transversely plicated lappets. The lateral sucking disks are modified as the larva matures so that they become lappets and come to lie within a cup-shaped hollow. Even at an early stage the larva is encysted.

The excretory system consists of a very truncate common vesicle and two long vesicular tubes. In the region of the transverse lappets these trunks give off a transverse tube which joins the two lateral systems. On its anterior side are given off tubules in fan-shaped arrangement. Lateral to the transverse trunk, and extending posteriad, are numerous anastomoses.

The genital cell-masses bespeak a typical holostome system. The yolk glands consist of paired tubular chords extending from the forking of the gut to the testes. They have large vesicular cells. Thick ducts lead into the ootype which is ventral to the ovary. The uterus leads obliquely from the right side of the ovary posteriad into the genital pouch. The testes are large pyriform glands, lying at the sides of the genital pouch. They open into the cone near the genital pore.

DISTOMATA

Distome cercariae may be grouped according to certain larval characters, which, altho not holding over to the adult trematode, are coexistent with other characters that are more deep seated. The Bitter Root species of the distome larvae consist of six xiphidiocercariae, two echinostome cercariae, and two furcocercariae. Among the xiphidiocercariae were found six species.

CERCARIA CRENATA nov. spec.

[Figures 5 and 10]

A heavy infection of this species was found in 13.6 per cent. of *Lymanaea proxima* Lea collected at the springs at Fort Missoula, Montana, in October, 1916. It is a minute larva, oblong-ovate in contour. The length of the trunk is 0.25 mm. and the width 0.13 mm. A weak tail, 0.15 to 0.16 mm. in length by 0.02 to 0.03 mm. in cross section at the base, is inserted into an aspinose caudal pocket, just posterior to the excretory vesicle. The larva possesses a very acute stylet fastened into the dorsal roof of the oral sucker, about 30μ long and 5μ broad at the base (Fig. 10).

The cercaria develops from the germ balls proliferated from the localized germinal epithelium within the very simple oval sporocyst. The mature sporocyst measures about 0.5 by 0.25 mm. It is non-muscular and has no organs of attachment. It depends for movement on the movement of the cercariae developing within it.

The prominent muscular parts of the larva are the large oral sucker, 60μ in diameter, the small acetabulum, 30μ in diameter, the small but

powerful pharynx with a median transverse constriction, and the crenate muscular excretory bladder.

Above the vesicle the excretory trunks diverge as a U from a single stem, each arm giving off a posterior and two anterior tubules.

The digestive system consists of a filiform esophagus and two ceca in the form of a typical furculum. The oral pocket anterior to the esophagus is large and deep. The pharynx sphincter surrounds the posterior half of the esophagus. When at rest the ceca end at the posterior margin of the acetabulum. Salivary glands consist of two series, an outer group of eight small cells and an inner group of five large cells. These groups empty into the oral cavity thru separate ducts.

The genitalia are represented by cell masses in the acetabular and postacetabular regions of the body. Antero-sinistral is Laurer's canal and proceeding forward is the coiled uterus-vagina fundament, ending in the genital pore mesad and just antieriad to the acetabulum. The testes are elongate pyriform bodies, extending postero-laterad at a 40° angle. The vitellaria are poorly developed, altho a few follicles and three main ducts are visible as they proceed mesad toward the ootype. The vitellaria are probably limited in the adult to the third quarter of the body.

CERCARIA GLANDULOSA nov. spec.

[Figures 11 and 16]

This species was obtained from a heavy infection of liver tissue of *Physa gyrina* Say from the vicinity of Hamilton, Montana, in October, 1916. The cercaria is moderately small, with a length of 0.45 mm. and a width of 0.2 mm. The tail has a length of 0.35 mm. and is 0.05 to 0.06 mm. in trans-section at the base. It is set into a caudal pocket, with locomotor spines in the lateral pockets. The stylet is placed in the roof of the oral sucker. It has a blunt point and measures 39μ in length by about 5μ in width (Fig. 11).

The sporocyst is extraordinarily simple in structure with a delicate epidermal wall. It is obovoid and measures 0.34 mm. in long diameter by 0.17 mm. in short diameter. The cercaria is proliferated from a localized germinal epithelium.

The cercaria is characterized by an unusual supply of glands. Cystogenous glands fairly crowd the other body structures. A paired series of nine glands of salivary nature empties into the oral cavity. In addition, the entire digestive tract is covered with gland cells, especially in the region of the muscular pharynx, so that the alimentary tract simulates superficially a cluster of grapes. The esophagus and the crura are short, just clasping the anterior margin of the acetabulum.

The oral sucker is somewhat larger than the acetabulum; the former measures 86μ in diameter and the latter 66μ .

The excretory system consists of a compressed vesicle and two cornua, each of which receives a single posterior tube and a single anterior tube. The anterior tube has three tributaries in the region of the acetabulum. Posterior to this region it receives several transverse tributaries (Fig. 16).

The genitalia are typically Plagiorchid. The ovary is situated dorsal to the acetabulum and merges into a large uterus-vagina fundament. Laurer's canal is prominent, arising from the vicinity of the ovary and turning dorso-sinistrad. The testes are not distinguishable at this time. The vitelline follicles extend from the extreme oral region to the extreme posterior region. Vitelline ducts run mesad toward the region of the ovary.

CERCARIA DIAPHANA nov. spec.

[Figures 12 and 17]

The species *Cercaria diaphana* is a delicate larva of such a beautiful gray as to remind one of a mere shadow. It is extremely transparent. It occurred as a heavy infection in the liver tissues of *Lymnaea proxima* Lea obtained from the Bitter Root River, Corvallis, Montana, in October, 1916. When contracted the larva is compressed ovoid, and measures 0.2 to 0.26 mm. in length and 0.1 to 0.12 mm. in width, but it is capable of extraordinary expansion. The tail is lanceolate, 0.15 mm. in length by 0.04 mm. in trans-section at the base, where it is included within the spinose caudal pocket.

The sporocyst in which the cercaria develops, is oblong, measuring 0.35 by 0.15 mm. One end may be drawn out as a sort of club-shaped process. An extremely simple germinal epithelium produces the cercariae. It is non-localized and lines the whole body cavity. No external organs of attachment or movement are present.

The oral sucker of *Cercaria diaphana* measures 44μ in cross section, and the acetabulum only 32μ . The tail is deeply sunken at the base into the posterior caudal pocket. There are a few (eight to ten) long spines at the dorsal edges of the pocket. A unique stylet is located in the dorsal roof of the oral cavity. It measures 39μ in length by 5μ in breadth at the base. Its anterior reinforcement is confined to two dorso-lateral plates at the anterior end. Between these lies a minute spine 5μ in length by 0.5μ in diameter (Fig. 12).

The excretory system consists of a highly muscular, compressed vesicle, from which there extends antieriad a long median protuberance. This trunk forks to form two trunks slightly posteriad to the acetabulum. Just postacetabular each trunk becomes constricted and connects

with a common lateral tubule. The tubule receives three main branches, two from the cephalic region and one from the caudal portion (Fig. 17).

The digestive system consists of a long slender esophagus and crura of equal length. The latter are broadly furcate. A small muscular pharynx is provided with an immense mass of gland cells. The pharynx itself measures about 15μ in cross section, while the gland complex includes a sphere of 65μ diameter. In addition, there are the paired salivary glands, eight in each paired group, small and poorly developed, emptying into the oral pocket.

The genital cell masses are typically Plagiorchid. Vagina, Laurer's canal and ovary are situated dorsad to the acetabulum. Testes are not yet visible. Vitelline follicles extend from the posterior margin of the oral hood to the base of the caudal pocket. Ducts arise from the ovary posterior and lateral, and are directed antero-mesad.

CERCARIA DENDRITICA nov. spec.

[Figures 13 and 18]

The species *Cercaria dendritica* was obtained from the liver tissues of highly infected *Lymnaea proxima* Lea, collected from the sloughs of the Bitter Root River at Fort Missoula, Montana, in October, 1916. The larva is an extremely muscular individual, altho the tail is weak and of questionable value in movement. The cercaria performs a characteristic "measuring worm" movement as it travels forward. It is about 0.38 mm. long by 0.15 mm. wide, and has a tail 0.16 by 0.04 mm. at the base, inserted into a spinose caudal pocket.

The oral and ventral suckers are large and well developed. They measure 62μ and 60μ , respectively, in diameter. The tail is included at its base within a caudal pocket provided with stout spines thruout the entire lining. The stylet in the roof of the oral cavity measures 44μ in length by 14μ in breadth thru its basal knob. The quill is triangular, scutate, and is joined to the shaft by a median and a pair of lateral reinforcements (Fig. 13).

The sporocyst is well developed. It consists of an elongate ovoid body provided with an oral sucker 80μ in diameter and is well supplied with muscular elements. The sporocyst itself measures 0.38 by 0.11 mm. The germ cells are situated at the posterior end. The cercaria is obovate, possibly due in part to the extreme muscular development of the oral sucker.

The excretory system deserves special emphasis. The sub-spherical crenate vesicle is remarkably muscular and the two cornua which are anterior are equally muscular. At the extreme anterior reaches of each cornu three tubules flow into it, two from the anterior portion and one from the posterior extremity. The tubules are dendritic (Fig. 18).

The digestive tract consists of a large pharynx 30μ in transection and 36μ long, a short esophagus of about two-thirds the length of the pharynx, and extremely rudimentary crura, hardly as long as the non-muscular portion of the esophagus. Salivary glands, eight in number on each side, arise from the region just anterior to the oral cavity.

The genital organs are well-defined. Ovary and uterus lie on the right side over the acetabulum. On the left side is the definitely outlined Laurer's canal, and just caudad to the acetabulum are the testes. Yolk glands consist of a pair of rather slender racemes arranged in zigzag fashion all along the lateral reaches of the cercaria, from the extreme ends of the trunk. The vitelline ducts lead into the ootype from a posterior angle.

Cystogenous cells fill all of the mesenchyme spaces of the body. They are large, white, oval bodies. All of the cercariae reach maturity almost synchronously. They are mature when they break thru the wall of the sporocyst and swim out into the surrounding medium. The tail is soon cast off. In fact, the animal travels much more rapidly without the tail than with it, for it can then use the spines of the caudal pocket. Encystment is slow; the cyst is a thin oval membrane within which the larva is coiled.

CERCARIA MICROPHARYNX *nov. spec.*

[Figures 14 and 19]

This species was secured from the liver tissues of *Lymnaea proxima* Lea obtained from the Rattlesnake Creek, Missoula, Montana, in November, 1916. The cercaria is oval, minute, measuring 0.18 mm. in length by 0.09 mm. in width. The tail is 0.14 mm. long by 0.03 mm. in width at the base. It is fairly active.

Anteriad is the stylet organ, superficially set in the oral roof, so that its leverage is poor. The organ is rounded at the point, and reinforced all around the margin. Across the top is a thin translucent mucoid velum. The stylet is 34μ long and 5μ in breadth along the shaft (Fig. 14). The tail is inserted proximally into the caudal introvert provided with spinose projections. The entire body is covered with minute spines arranged in diamond pattern and decreasing in size from the anterior to the posterior margin.

The excretory system is entirely non-muscular. The vesicle is sub-spheroid and laterally compressed, and the two cornua which arise antero-laterad are likewise sub-spherical. Each receives three tubules, a small posterior, a large outer, and a small inner anterior tubule (Fig. 19).

The digestive tract is diminutive. It consists of a minute pharynx around the middle portion of the esophagus, and small vesicular crura

Paired groups of salivary glands, each with eight cells in the group, are found in the acetabular region. The pre-pharynx is provided with a large spheroidal group of small gland cells.

The genital cell masses consist of a non-differentiated band of tissue just dorsal and posterior to the acetabulum, in the neighborhood of the future ootype, a uterus-vagina cell mass running cephalad over the acetabulum, and in addition broad bands of yolk follicles extending along the margins from the pharynx region to the caudal pocket. The beginning of the tests are not yet distinguishable. Laurer's canal is definitely set off to the right of the uterus.

The sporocyst is ovoid, measuring 0.24 by 0.18 mm. It is remarkably simple, with a single layer of epidermal cells constituting the body wall. The germinal epithelium is non-localized. There is an intercellular complex of excretory channels in which are found many excretory calculi. When the germinal epithelium has been exhausted, the cercariae maturing last drop off their tails and encyst within the sporocyst. The cercaria is provided with many minute subspherical cystogenous cells thruout the parenchyma.

CERCARIA RACEMOSA nov spec.

[Figures 15 and 20]

This ornate cercaria was found in the liver tissues of *Lymnaea proxima* Lea obtained from the sloughs at Fort Missoula, Montana, in October, 1916. It is oblong-spatulate, with a delicate quill stylet and a fluted tail. The body measures 0.29 mm. in length by 0.11 mm. in width, while the tail is 0.22 mm. in length by 0.04 mm. in width at the base.

Cercaria racemosa is found developing in rhomboidal sporocysts about 0.93 mm. long and 0.56 mm. in trans-section, with a poorly defined attachment pocket at one end. At the antipodal end is the localized germinal epithelium from which the cercariae develop. The cercariae grow to maturity within the sporocyst.

The body of the cercaria is aspinose. The slender stylet measures 12μ in length by 2μ in width at the base (Fig. 15). This is reinforced only at the pointed tip. It is advantageously set in the roof of the oral cavity so as to give a good leverage.

A pair of non-pigmented eye-spots are present superficially in the region of the brain ganglia.

The excretory organs consist of a truncate vesicle, a median tube anterior to the vesicle, and two fusiform cornua which receive racemose tubules at their anterior extremities. The vesicle contains two groups of three cells each, probably glandular, attached to the anterior margin of its inner wall (Fig. 20).

The digestive tract consists of a small muscular pharynx, a long slender esophagus, and short crura clasping the anterior margin of the acetabulum. Paired salivary glands, eight in each group, are situated in the acetabular region. Their long ducts open into the oral cavity. The genital cell masses are restricted to the acetabular and post-acetabular portion of the cercaria. A vagina and a Laurer's canal are discernible. Vitelline glands are confined to the region just posterolateral to the ootype. No testes can be made out.

The tail is of considerable power in swimming and is not readily detached. No encystment occurs for some time after the cercaria is placed in a watch glass of normal saline solution.

ECHINOSTOME CERCARIAE

Echinostome cercariae possess a circum-oral collar with spines and usually contain three large flame cells in the anterior portion of the excretory system. The further criteria added by Cort (1915:37), namely, an excretory system opening on each side of the anterior part of the tail, and "tail powerful, longer than body," may or may not be typical of individual species: they are not family characters. Of the two species of this family that have come under the writer's observation, only one has a tail longer than the trunk, while neither one has the excretory system opening on each side of the anterior part of the tail.

The two species described by Cort (1915) as echinostome cercariae, *C. trivolvis* and *C. rubra*, with the probable echinostome larva, *C. reflexae*, constitute the only species of this group previously described from North America. Two new species are contributed from the Bitter Root collection.

CERCARIA TRISOLENATA nov. spec.

[Figure 6]

This species is an echinostome larva of unusual features. It is considerably more slender than the usual species in this group. The tail is short and lanceolate. The acetabulum is studded with spines. The body is 0.45 mm. long and 0.1 mm. wide when the animal is at rest. The tail measures about 0.2 mm. in length and 0.016 mm. in section at the base. The acetabulum is a third larger in diameter than the oral sucker which measures 30 μ . Around the dorsal margin of the collar and extending a short distance ventrad is a ring of spines, 36 in number, in a single, altho somewhat irregular line. These spines are aciculate, yet blunt at the base and at the extreme tip.

The cercaria is developed in rediae found in the liver tissues of two snails, *Physa gyrina* Say and *Planorbis trivolvis* Say, collected along the entire course of the Bitter Root River. It is one of the two dis-

tinctly cosmopolitan species of the valley. While the infection of the Physa was heavy (22 to 100 per cent of all Physas examined) and the Planorbis infection was 50 per cent, the infection of the individual Planorbis was much heavier than that of the individual Physa.

The redia when mature measures 1.0 mm. in length by 0.25 mm. in cross-section. It is provided with a small pharynx, 55μ in trans-section, and a large rhabdocoel gut extending the entire length of the body cavity. The locomotor "feet" occupy a position about one-third the body distance from the oral opening. Proliferation of germ balls occurs from the posterior end. The rhythmic movement of the redia is due to its own muscular action and that of the daughter cercariae.

The excretory system of the cercaria consists of a small obtruncate bladder and the lateral canals which remain unbranched until they reach the cephalic region. Here each forms a single deltoid anastomosis and end in three flame cells. The tubules are filled with excretory granules. The caudal tube is single, median, and unbranched thruout the entire course.

The digestive tract consists of a long esophagus with a small pharynx mid-way along its length, and a pair of long crura extending posteriad to the subterminal region. Soon after the crura arise from the esophagus they cross under the excretory trunks and run parallel to them externally all the way posteriad.

The genitalia are not well developed in the larva. They consist of an ovarian mass some distance behind the acetabulum, a vagina to the right and just anterior to the acetabulum, and two testes, one behind the other in the posterior extremity of the trunk.

Encystment starts with the rejection of the tail and later the slow formation of a semi-membranous cyst capsule from the abundance of glandular material with which the cercaria is filled. The cyst is very transparent, but extremely resistant to mechanical and chemical disturbances. The trisolenate arrangement of the excretory tubules cephalad is clearly seen thru the cyst membrane.

CERCARIA BIFLEXA nov. spec.

[Figure 7]

This form is broadly wedge-shaped at the cephalic margin and rounded posteriad, with long powerful tail, large groups of salivary glands and marked bodily activity; the cercaria is extraordinarily destructive to the host which harbors it. It was found in a small percentage of *Physa gyrina* Say from the vicinity of the Buckhouse Bridge near Fort Missoula in November, 1916.

The collar spines are elongate-ovoid, 10μ in length, 42 in number. The acetabulum is situated in the posterior third of the trunk. It is 60μ and the oral sucker 50μ in diameter. The body measures 0.45 to

EXPLANATION OF PLATE

Fig. 1.—Dorsal view of *Cercaria pellucida*; specimen partially contracted, showing eye-spots, excretory and genital systems. $\times 80$.

Fig. 2.—Dorsal view of *Cercaria konadensis*; specimen relaxed, showing eye-spots and anterior pigmentation, excretory and genital systems, and gland cells of tail. $\times 105$.

Fig. 3.—Ventral view of *Cercaria ptychocheilus*; specimen freed from cyst, showing digestive, excretory and genital systems. $\times 80$.

Fig. 4.—Ventral view of *Cercaria flabelliformis*; young specimen within cyst, showing digestive ceca, excretory system and lateral suckorial cups. $\times 50$.

Fig. 5.—Dorsal view of *Cercaria crenata*; digestive, excretory and genital systems shown; salivary glands in two series, inner and outer, empty into oral cavity thru long ducts; cystogenous cells not shown. $\times 170$.

Fig. 6.—Ventral view of *Cercaria trisolenata*; digestive and excretory systems shown. $\times 150$.

Fig. 7.—Ventral view of *Cercaria biflexa*; excretory and genital systems shown. $\times 105$.

Fig. 8.—Posterior two-thirds of *Cercaria gracillima*; specimen shows genital cell masses; testicular follicles proliferated from the posterior end. $\times 270$.

Fig. 9.—Dorsal view of *Cercaria tuberistoma*; excretory system and salivary glands shown. $\times 170$.

Fig. 10.—Stylet organ of *C. crenata*. $\times 540$.

Fig. 11.—Stylet organ of *C. glandulosa*. $\times 370$.

Fig. 12.—Stylet organ of *C. diaphana*. $\times 540$.

Fig. 13.—Stylet organ of *C. dendritica*. $\times 250$.

Fig. 14.—Stylet organ of *C. micropharynx*. $\times 540$.

Fig. 15.—Stylet organ of *C. racemosa*. $\times 333$.

Fig. 16.—Excretory vesicle of *C. glandulosa*. $\times 75$.

Fig. 17.—Excretory vesicle of *C. diaphana*. $\times 170$.

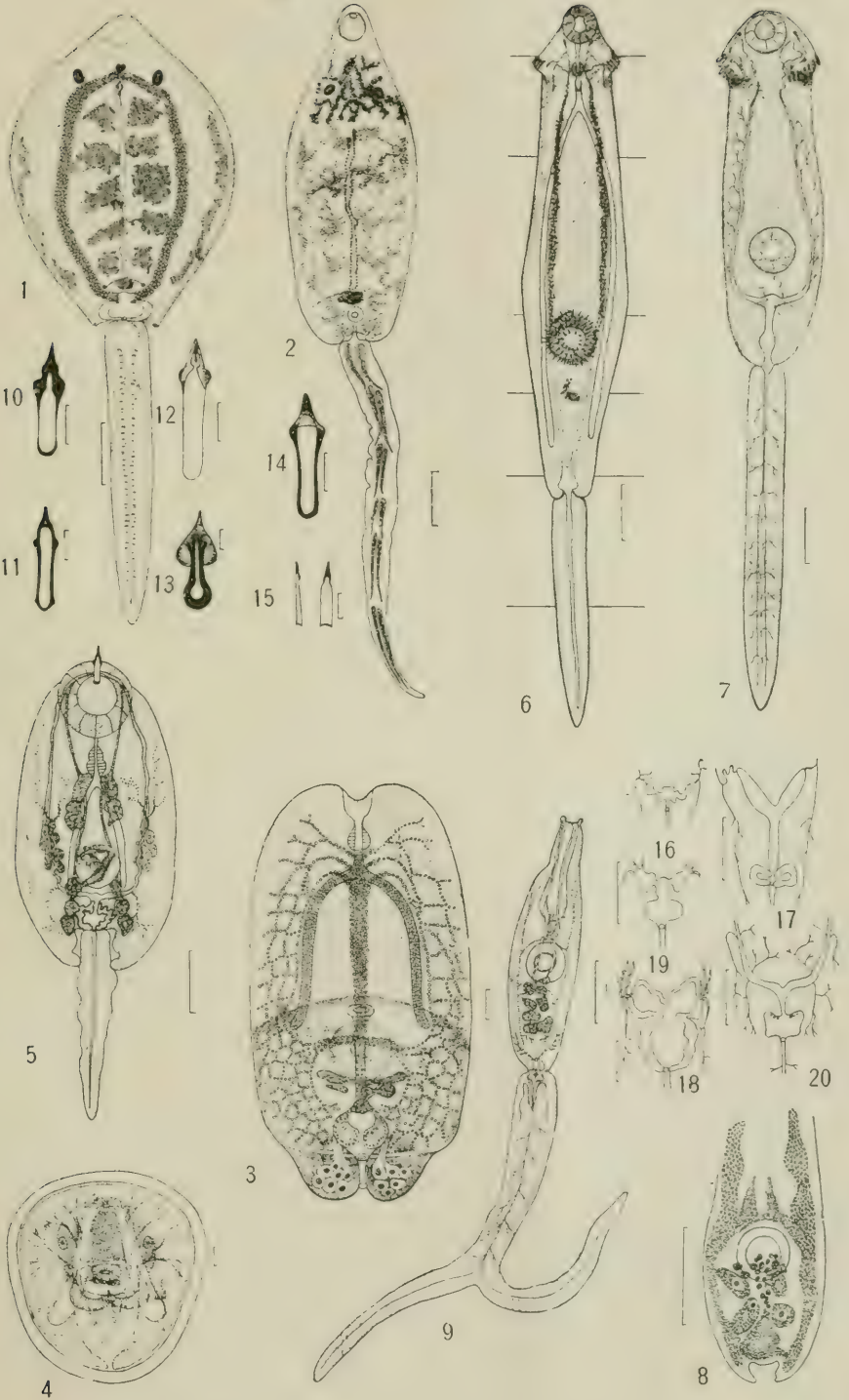
Fig. 18.—Excretory vesicle of *C. dendritica*. $\times 113$.

Fig. 19.—Excretory vesicle of *C. micropharynx*. $\times 270$.

Fig. 20.—Excretory vesicle of *C. racemosa*. $\times 150$.

Reference line in Figs. 1-9 and 16-20, 50μ long; in Figs. 10-15, 10μ long. Lines in Fig. 6 indicate important regions not discussed in this paper.

PLATE



0.5 mm. in length by 0.13 to 0.15 mm. in width. The tail is of equal length to the body and 0.06 mm. in cross-section at the base.

The redia of *Cercaria biflexa* measures 0.4 mm. in length and 0.09 mm. in cross section. The locomotor "feet" are found in the posterior third of the body. The pharynx is moderately large, 40μ in cross section, and well developed. On the other hand, the rhabdocoel gut is short, extending only thru the cephalic fourth of the body. The posterior margin is characterized by a number of small integumentary spines. Cercariae are produced from a localized germinal epithelium in the posterior part of the body.

The excretory system of the cercaria consists of an elongate vesicle and a U-shaped trunk system leading into it anteriorly. Lateral tributaries are received by these two branches thruout the body tissues. At the anterior end cephalad to the collar prominence, the main tube on each side becomes attenuated, loops back on itself as far as the collar region, then turns again antieriad and ends in three flame cells. The excretory tube in the tail is single, two-fifths of the distance distad. There it forks, altho the bifurcations never open laterad.

The digestive tract consists of a very long esophagus, extending to the acetabulum, and a pair of ceca arising just preacetabulad and extending nearly to the posterior margin of the body. An inner and an outer series of salivary glands, fifty to sixty in each series, occupies the larger part of the body ventral to the excretory trunks. They empty thru united lateral ducts into the oral cavity.

The genital cell masses are fairly well developed in the cercaria. An ovary posterior to the acetabulum, vitelline ducts and a uterine duct have a common center at the ootype. The uterus proceeds antero-dextrad around the acetabulum and ends in a large muscular vagina anterior to the acetabulum. Two testes are observable posteriad to the vitelline ducts, one almost on top of the other. The animal encysts readily. While no encystment was noticed within the redia, it may take place as soon as the cercaria escapes from the mother. Most of the specimens were found encysted in the tissue of the host.

THE FURCOCERCARIAE

This group of larval trematodes is characterized by a forked tail and, as far as the writer knows, the absence of a true pharynx. However, glands in the pharyngeal region may lead one to consider the mass a pharynx, which is evidently the error Looss (1896) has made in his study of *Cercaria vivax* Sons. The apharyngeal furcocercariae are undoubtedly larval Schistosomidae, as demonstrated by the experimental work of Leiper (1916) and by a close comparative study which the writer has made on larvae and adults. Two new furocercous larvae

have been obtained from the Bitter Root Valley. These, in addition to *Cercaria douthitti* (Cort, 1915), constitute the only described forms of North American Schistosome larvae.

CERCARIA GRACILLIMA nov. spec.

[Figure 8]

This is an extremely slender tho wiry individual. It has a body length of 0.13 to 0.16 mm. and a width of 0.02 to 0.03 mm. The tail is approximately twice as long as the body and is equally divided between the simple and bifurcate portions. This cercaria is of common occurrence in the Bitter Root Valley, altho it is most abundant in the lower part of the valley. It was found abundantly in liver tissues of *Physa gyrina* Say, and in *Lymnaea proxima* Lea, along with a large infection of *Cercaria micropharynx*.

The body is provided with an oral sucker covered with spines; the ventral sucker measures about 12 μ . The oral sucker can be drawn into the esophagus. Vestiges of non-pigment eye-spots are found dorsally in close proximity to the brain.

The cercariae develop in sporocysts from a localized germinal epithelium. The proximal end is provided with an attachment disk. The sporocyst is about 0.5 mm. long at maturity and 0.025 mm. wide. It has no musculature and depends on the cercariae within for its motility. The cercariae escape thru a rent in the wall of the sporocyst.

The excretory system includes a common non-muscular vesicle at the posterior margin of the trunk, and two lateral canals which anastomose frequently and characteristically in the anterior two-thirds of the body. Flame cilia are present in a restricted region of the main tubes in the posterior third of the body. The junction of body and tail is accompanied by an "eyelet anastomosis," commonly found in furcocercous larvae. The common tube of the anterior unbranched region of the tail, branches into the rami of the tail.

The digestive system consists of a long esophagus which branches to form the ceca just anterior to the acetabulum. The ceca end at the posterior margin of the acetabulum. Paired salivary glands, four to each series, lying in the posterior third of the body, open by long ducts into the oral cavity.

Anterior to the acetabulum are the ovary-uterus cell mass on the right and that of the cirrus on the left. Posterior is the male germinal epithelium from which is proliferated a large number of testicular follicles. Ventro-lateral are lines of vitelline glands which empty their products thru ducts into the ootype anterior to the acetabulum.

Encystment has not been noted in the species.

CERCARIA TUBERISTOMA *nov. spec.*

[Figure 9]

Two prominent tubercles are present at the anterior end of the spineless body of this species. The chamber for the oral sucker occupies the core of the anterior third of the worm. The body is about 0.2 mm. long by 0.05 to 0.06 mm. wide. The tail is about 0.32 mm. long, of which the unbranched portion constitutes approximately one-half. It measures 35μ at the base. The ventral sucker measures 30μ . The larva was found in *Physa gyrina* Say at Corvallis, Montana, in October, 1916. The infection was light.

The cercaria develops in sporocysts, which are about 0.5 mm. long and 0.05 mm. in trans-section. At one end is a sucking disk, and at the other end is the broad attachment organ. The germinal epithelium is localized at this latter end.

The excretory system consists of a small muscular maliform bladder situated posteriad, and slender lateral trunks which receive occasional branches more anteriad. No flame cell areas have been made out. The "eyelet anastomosis" at the junction of the body and tail is muscular. A slender median caudal canal divaricates just anterior to the bifurcation of the tail. At the proximal end of the tail are given off a pair of lateral tubules which are recoiled on themselves.

The digestive system is of the usual type for the furcocercariae. The genital anlagen have not been worked. Encystment has not been observed in the species.

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THE DEVELOPMENT OF GREGARINES AND THEIR
RELATION TO THE HOST TISSUES: (I)
IN *STENOPHORA LACTARIA*
WATSON *

MINNIE WATSON KAMM

The object of this paper is the depiction of the stages through which the sporozoite passes in the species *Stenophora lactaria* Watson in becoming a free adult sporont. Whether or not the trophozoite at any stage in its development possesses an epimerite and the effect of the parasite upon the cell parasitized will also be discussed, conclusions reached affecting only the particular species under consideration. This effect upon cells parasitized will be reported in several other genera before conclusions can be stated as to the general influence upon the host-cells.

Stages in the growth of the parasite from the sporozoite to the sporont have been described by many writers. Léger and Duboscq have studied the development of Pyxinia (1902), Stylorhynchus (1904), and Pileocephalus (1909a), and to some extent of Stenophora (1904); Laveran and Mesnil of Gregarina (1900); and Siedlecki, Brasil, Caulery and Mesnil, and Hesse of parasites in the tunicates and annelids. In very few instances has a complete series of stages been shown.

To the writer's knowledge, consecutive stages from the incipient penetration to the vacation of the cell by the parasite have not been depicted for the genus Stenophora. I am able to offer nine stages, somewhat arbitrarily chosen, in the development of a single species, the species chosen being *Stenophora lactaria* from the milliped *Callipus lactarius* (Say), described by the writer (1915: 29-30; 1916: 72-4). The intestines of several hosts were removed intact, fixed with corrosive-acetic, and sectioned. Sections were cut 4μ thick and stained with Ehrlich's hematoxylin. All the intestines proved heavily infected. The lumen reveals parasites in the proventriculus and in the large intestine, but intracellular stages are generally lacking except in the first-named portion.¹ In several instances parasites were found boring through the walls into the coelom. Successive movements have been traced from the penetration of the muscular layer of the digestive tract

* Contributions from the Zoological Laboratory of the University of Illinois under the direction of Henry B. Ward, No. 88.

¹ The digestive tract of the milliped (Fig. 12) is, according to Leidy (1853), divided into (a) and (b) six salivary glands, (c) esophagus, (d) proventriculus, (e) two bile ducts, (f) broad opaque cuticular curtain, (g) ventriculus, (h) large intestine, (i) rectum.

through the coelomic epithelial layer until the parasites are free in the coelom. This phenomenon was reported by the writer in a former paper (1916:29). The gregarine possesses no boring apparatus; it has no chitinous style and it even lacks an epimerite; thus, mechanical apparatus being absent, the hypothesis is made that the means used is chemical and that the body of the parasite secretes a fluid destructive to the cell epithelium. The cells in the immediate vicinity of the tubular opening are crowded back and disorganized and their nuclear material scattered. Both the nuclear and cytoplasmic protoplasm stain less deeply than the normal. This fluid present is either a secretion of the parasite available for purposes of penetration or the normal excretion of the parasite which is toxic to the adjacent tissues.

After the cyst has been discarded from a host along with its feces and has dehisced, the spores are liberated and accidental parasitism occurs, the released spores being eaten by a host of the same or a nearly related species,² even by the original host. The spore, upon reaching the alimentary canal, loses its sporocyst by the action of the digestive juices, releasing, in all the Eugregarinae, eight falciform sporozoites.

The stages in development from the incipient to the mature parasite which have been studied are as follows:

The liberated sporozoite, which is slightly motile, although it possesses neither cilia nor flagella, reaches the epithelium of the proventriculus and penetrates between the terminal cilia into the free end of one of the absorptive cells (Fig. 1). The sporozoite can be readily detected, although less than 5μ in length, because of its intense coloration, staining darker than the cytoplasm of the cells. A nucleus can be discerned as it is still darker. The sporozoite loses its falciform shape and rounds off at one end, penetrating the cell by the remaining pointed extremity.

It journeys down the cell past the nucleus, the pointed end preceding (Fig. 2).³ The parasite is small enough to make the passage without injuring the cell other than by the temporary crowding of the nucleus.

It comes to rest at the end of the cell, next the muscular layer (Figs. 3 and 4, a). The sporozoite now becomes trophic, viz., a *trophozoite*. With the beginning of growth and consequent excretion, the parasite effects a chemical change upon the parasitized cell. The cytoplasm becomes slightly vacuolated and stains a little less deeply than normal, but its nucleus is not yet visibly affected.

In the next stage, the parasite has lost its characteristic sporozoite shape and has become a subspherical body of larger dimensions and

² Léger and Dubosq (1902) have shown that if the spore is eaten by any other animal, it will not dehisce but passes intact through the digestive tract.

³ Léger and Dubosq record that in *Stenophora aculeata* the sporozoite pushes the cell nucleus ahead of it toward the muscular layer gradually absorbing it.

with a conspicuous nucleus containing one karyosome, which is characteristic of the adult nucleus (Fig. 4, b). The host cell has become still further vacuolated and stains still less deeply. The nucleus is now affected, for it, too, stains more faintly.

The trophozoite now becomes differentiated into protomerite and deutomerite separated by a septum (Fig. 4, c). The nucleus and karyosome have grown more rapidly than the parasite and are proportionally much larger than before. It is already apparent that the protomerite stains more intensely than does the deutomerite. This continues into the mature sporont stage. The young trophozoite generally orients itself so as to lie "head" downward, i. e., with the protomerite next the muscular layer, but exceptions occur. In some instances also the parasite lies at right angles to its usual position or in the antero-posterior plane of the host. In a few instances out of hundreds, the protomerite was seen to be directed toward the lumen. Léger and Duboscq record this in *Stenophora aculeata* (1904), and Mercier in *Cephaloidophora talitri* (1912). The former authors name the two possibilities, viz., that the sporozoite penetrated the cell posterior end first or else turned after entrance. The cytoplasm of the cell is still further destroyed. It may be vacuolate with the nucleus intact and seemingly but little changed (Fig. 5) or vacuolate with the nucleus already destroyed, its remaining chromatin massed at the base (Fig. 4, c).

The protoplasm of the two divisions of the trophozoite is becoming differentiated (Fig. 6). In the protomerite it stains deeply and consists of small homogeneous granules, while in the deutomerite it is more coarsely granular and less closely packed together. The nucleus of the parasite has now begun to assume the shape of that in the adult sporont; it has become ellipsoidal and is now smaller in proportion to the size of the gregarine than it has been before. The protomerite has acquired a papillate apex which is retained in the adult. This is the only structure in this species which may be compared to an epimerite. It is not a true epimerite, for it performs no function. It may possibly be a vestigial remnant from a lower group of gregarines which possesses and uses a true epimerite, but the relationship of the families of gregarines has been little discussed and the higher or lower position of the Stenophoridae is not determined. Some members of the genus appear to possess epimerites, as *S. nematoides* Léger and Duboscq (1904) and *S. dipolcorpa* Watson (1916); one species retains a minute apical style (*S. aculeata* Léger and Duboscq (1904)); but most species have no trace of an epimerite at any stage of development.

The parasite has by this time acquired something of the normal sporont shape (Fig. 7). It has grown so as to absorb several adjoining cells besides that first parasitized, nuclear and cytoplasmic vestiges

remaining at the base at one side of the apex of the protomerite. By growth and expansion the animal has laterally compressed the cells which border it, leaving a small opening into the lumen of the alimentary tract. It is my opinion that a part of the parasite's nourishment is acquired by absorption direct from the intestine through this opening.

The stage shown in Figure 8 is very similar to the last. The space leading to the lumen is larger and the contiguous cells have been forced farther apart with a consequent compression of many cells, their sub-spherical nuclei having become very long and slender. It is to be noted that the deutomerite is growing faster than the protomerite and is forcing itself down over the former at the septum, while the protomerite is flattened against the muscular layer.

The trophozoite has become capable of living free in the intestinal lumen (Fig. 9). It no longer receives sufficient nourishment from the epithelium and through the small opening into the lumen, and is forced out henceforth to lead a free existence in the lumen. Just what forces it out of the epithelium, I am unable to say. One would not be inclined to assume in it the power of volition with the ability to leave the cell at the critical moment; but, on the other hand, one cannot assume that the cells force it out by swelling and expressing it, for up to now they have been passively forced apart by the growing parasite.

By whatever means, the animal has left, after absorbing nourishment from many cells which are not entirely destroyed but only distorted with their nuclei shrivelled. These cells are probably able to revive themselves and acquire new vigor, unlike the first few cells, which were totally destroyed. The animal has not straightened itself out yet from the cramped position when embedded, and the deutomerite still overlaps the protomerite. The liberated trophozoite has become a free living *sporont*.

The young sporont (Fig. 10) must now receive all its nourishment from liquids in the alimentary tract and not indirectly through the media of cells. The animal is rotund in appearance and sluggish; the epicyte is seen to be thicker at the septum than elsewhere; the papillated apex of the protomerite is apparent, and there is visible for the first time a minute indentation in this apex which is frequently reported for this genus.

The fully developed sporont is much more graceful (Fig. 11). The deutomerite has grown more rapidly than the protomerite, leaving the latter a small conoidal segment while the latter has elongated and become slender and tapering. The nucleus acquired its permanent shape in an early stage, and now remains elongate-ellipsoidal with one large karyosome.

It is seen that in the species considered there is no epimerite. Nourishment, then, takes place by absorption from surrounding cells directly through the epicyte of the parasite. An epimerite would, moreover, be superfluous to an animal which is intracellular in development, useful only in a species in which one end only of the parasite is embedded in an epithelial cell.

The parasitized cell is apparently unaffected until the sporozoite has begun to grow. Then the cytoplasm becomes reticulate, vacuolate, and resistant to the staining fluid. It commences to shrivel in length and in width; atrophy has set in. This may be due to the toxic influence of the parasite, but it is undeniably due in part at least to the absorption of its vital fluids by the rapidly growing parasite. No hypertrophy is noted from the first. The nucleus is affected less readily than the cytoplasm, but soon shows a resistance to the stain. For a time the chromosome count is unaltered, although the size of the individual chromosomes is reduced. The growing animal utilizes the space left by the contracting cell and compresses adjoining cells in its proximity, while the cells become elongate and wider at the uncompressed ends. The posterior end of the gregarine soon projects into the lumen, for it forces the cells so far apart that they are no longer able to overlap the end of the parasite. The latter now undoubtedly receives some of its nourishment from the lumen and the drain on the cells themselves is decreased. This probably accounts for the fact that frequently not more than two or three cells are actually destroyed, the others being compressed only during the occupancy of the parasite. When the latter departs, the cells return to something like their former shape, and the space through which the parasite left almost immediately closes over.

The cells surrounding the parasite always are separated a trifle from it, leaving in many instances a thin clear area around it. I think this is due to the fact that in fixing the parasite shrinks slightly more than does the host tissue, rather than to the fact that the parasite affects the cells toxically and causes them to draw back.

The cells of the proventriculus lie in lobes (Figs. 1, 4, 7, and 9), the deep-seated lobes being the ones generally parasitized. Only in unusual cases do the outlying ones harbor gregarines. The deeper seated cell forms a safe harbor for the young sporozoite where it is not in danger of being swept along in the lumen by the undigested food masses and by the animal's movements. These shorter cells also afford easy exit when the parasite is ready to leave the epithelium, being about as long as the mature trophozoite when it leaves.

The trophozoite always lies next the basement muscular layer, but not actually in contact with it; it never remains half way up the cell. As aforesaid, it is usually placed "head downward."

That the gregarine is intra-cellular rather than inter-cellular is seen from the early stages when the cell itself contains the parasite; with later stages alone this could not be determined.

Several writers have noted that in the species which they studied distinct hypertrophy of the parasitized cell occurred. Laveran and Mesnil (1900) and Léger and Duboscq (1909a) have shown that the parasitized cell decreased in length, but at the same time increased abnormally in width due to the influence of toxic excretions of the parasite. The latter authors think the cell is not killed, but assumes its normal shape and function after being relieved of its burden. They state, however, that the influence of the parasite upon the host cells is very different in different hosts, with different parasites, and even in different parts of the same host.

The present research has shown that there is no hypertrophy of the cell, but that the originally parasitized cell shrinks from the start without widening and is destroyed, and that the two adjoining cells are in many instances also destroyed. Other nearby cells are temporarily compressed and elongated, later to return to their normal functions; their staining reaction is unaffected.

SUMMARY

Consecutive stages in the growth of *Stenophora lactaria* Watson are depicted.

This species does not possess an epimerite.

Development is intracellular and the parasitized cell is destroyed.

No hypertrophy is indicated at any stage of development.

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EXPLANATION OF PLATES 1 AND 2

Fig. 1.—Sporozoite (*s*) beginning to penetrate an epithelial cell of the intestine.

Fig. 2.—Sporozoite (*s*) ascending the cell.

Fig. 3.—Sporozoite (*s*) at rest at the base of the epithelial cell.

The line at the right represents 50 μ .

Fig. 4.—Three stages in the growth of the parasite: (*a*) same as shown in Fig. 3; (*b*) trophozoite with enlarged nucleus and one karyosome; (*c*) larger trophozoite with formed septum, the cell nucleus destroyed and cytoplasm somewhat vacuolated. The line at the right represents 50 μ .

Fig. 5.—Still larger trophozoite with cell nucleus yet intact altho the cytoplasm is affected. The line represents 25 μ .

Figs. 6, 7, and 8.—Stages in the growth of the trophozoite, cell nuclei in each instance destroyed, vestiges remaining at the "head" of the parasite. The line represents 50 μ .

Fig. 9.—Trophozoite leaving the epithelium and migrating into the intestinal lumen. The line represents 50 μ .

Fig. 10.—Sporont soon after emerging and still rotund in appearance.

Fig. 11.—Mature sporont from lumen showing proportional growth of protomerite and deutomerite.

Fig. 12.—A copy of Leidy's figure (1853, Plate VII, Fig. 21) of the digestive tract of *Julus marginatus*.

PLATE 1

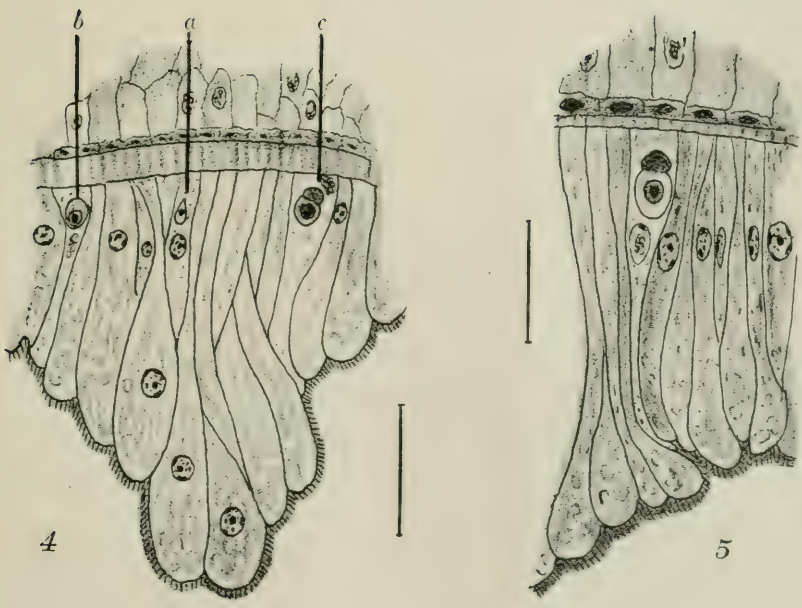
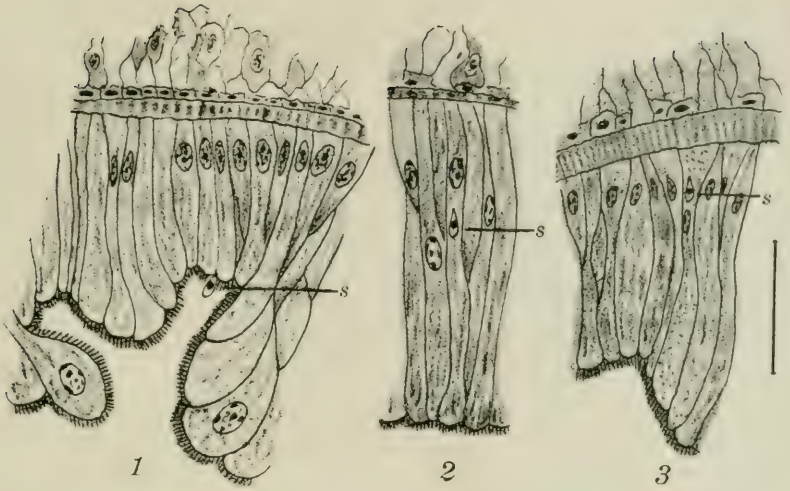
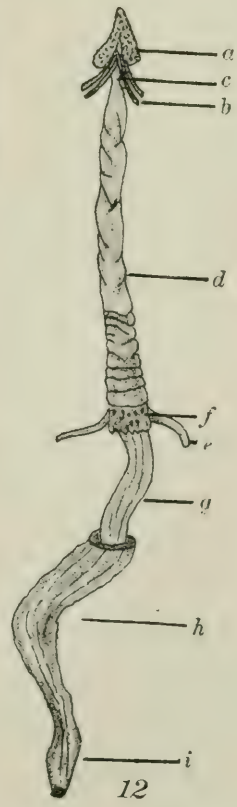
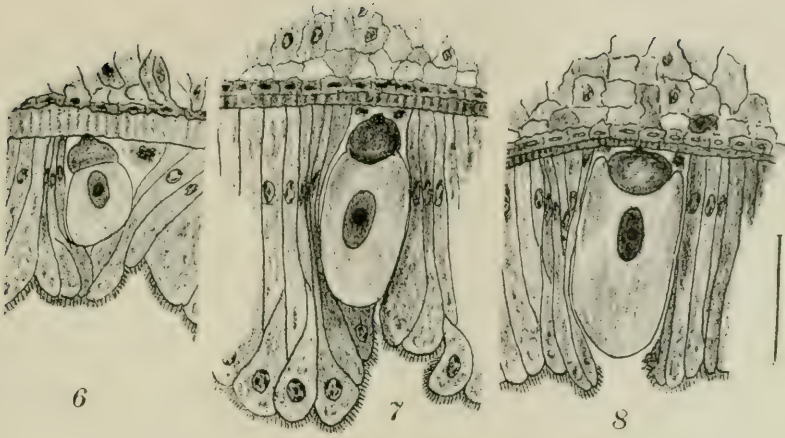


PLATE 2



THE CERCARIAE OF NATAL

F. G. CAWSTON

During the months of April, May, June, and July of 1916, I examined 1,500 molluscs from the rivers and fresh-water pools of Natal. They included several species. *Limnaea natalensis* is a common form with a dextral shell. *Physopsis africana*, a common mollusc amongst decomposing vegetation, has a blunt-pointed sinistral shell with a truncate columella. *Planorbis pfeifferi* is a common form with a round, flat shell. *Planorbis leucocheilus* is not unlike it, but is much smaller. *Isidora tropica* is fairly common; it has a blunt-pointed sinistral shell. *Isidora forskali* is rarer and has a conical shell. In one brickfield I found a large number of specimens of *Ancylus (ferrissia) burnupi*, which has a small oval shell.

Two hundred eleven specimens harbored cercariae of various kinds. Infected specimens were most common in one brickfield at Durban and in a small pool along the course of the Umsindusi River at Pietermaritzburg, which had been formed as a result of an overflow of the river. Infected specimens were most frequently met with in May and June. All of the cercariae possessed a long, slender tail; those that were found in specimens of *Physopsis africana* more often than not possessed divided tails. The tail was easily detached from the body of a cercaria and continued to move for some time after becoming free. All of the cercariae were distomes; the oral sucker was terminal, and in a few specimens the posterior border of the sucker was incomplete; the acetabulum was situated slightly nearer the tail end of the body. None of the cercariae had spines or stylets, and there were no projections from the body or tail. A pharynx was noted in only one form, and eye-spots were present only in cercariae from one specimen.

CERCARIA CATENATA

A large cercaria, *Cercaria catenata*, from the Toll Gate brickfield at Durban, present in about 30 per cent of *Planorbis pfeifferi* and in fewer *Limnaea natalensis* and *Physopsis africana* developed in rediae. These rediae gave an orange color to the liver-substance of infected specimens. The rediae were very mobile and possessed two pairs of locomotor appendages. The posterior extremity was pointed. There were infoldings of outer cuticula at the extremities of the posterior extremity of the redia and of its appendages which were not unlike suckers. The rediae contained a somewhat distended intestine,

a large amount of orange pigment, and several fully developed cercariae. On one occasion a cercaria was seen attempting to draw itself through the ruptured wall of the redia by means of its suckers. The head of the cercaria varied in appearance, but was often shaped like a leaf. It possessed a large oral sucker and a large acetabulum or ventral sucker; a chain of blackish granules, varying in number from about twenty-five to twenty-eight, lay on each side of the divided alimentary canal. The tail of the cercaria was as long or slightly longer than the body and tapered towards its extremity. Hypodermic injection of a large number of both rediae and cercariae into a guinea-pig threw no light whatever on the life history of this cercaria, which was the only form found to develop in rediae.

"TADPOLE" CERCARIAE

Sixteen sporocysts, containing leptocercous distomes, or "tadpole" cercariae, were found in *Physopsis africana* from the Umsindusi. Similar sporocysts were present in two specimens of *Limnaea natalensis* from the same source. The sporocyst intersected almost the entire liver-substance of infected specimens, giving it a whitish appearance. The cercariae consisted of a body with two suckers and a tail which was about the same length as the body. There was a divided gut and an elementary bladder. Some forms presented a stumpy appearance, others were longer. Some of these cercariae found in Durban suggested the appearance of Schistosoma cercariae in every respect except that their tail was not divided.

No cercariae were found in specimens of *Isidora* (over fifty were examined from infected places), and furcocercous cercariae were found only in specimens of *Physopsis africana*. Some specimens of this latter mollusc harbored more than one form of cercaria. Occasionally one came across a specimen which harbored both the "tadpole" and furcocercous forms.

Ninety-nine specimens of *Physopsis africana* harbored *Bilharzia* cercariae. These are characterized by the absence of a pharynx and by a divided tail. One specimen obtained from the Durban brickfields on May 9 harbored cercariae with long undivided tails, as well as a sporocyst containing an eye-spotted form of furcocercous cercaria. This is the only specimen of the kind I have seen, and, in consideration of its resemblance to the Egyptian form, I have suggested for it the name *Cercaria oculata*. The eye-spots had a crescentic appearance and were situated nearer the oral end of the body, on either side of the divided gut. They could be readily seen through the thin walls of the sporocyst in which the cercariae were well developed. No pharynx was discernible in the body of the cercariae. There was a long, slender tail which was divided into two short, fin-like prongs.

CERCARIA SECOBIANA

A common cercaria from the Umsindusi pool, for which I have suggested the name *Cercaria secobiana*, occurred in about seventy specimens of *Physopsis africana*. It was narrower and slightly smaller than the eye-spotted form. This distome had a long, slender tail which was divided into two prongs. The prongs were as long as the tail itself. When the tail moved, the prongs became bent to the form of a crescent, causing the cercaria to swim backwards—a form of locomotion which would seem to be common to furcocercous cercariae. The cercariae developed in a sporocyst which intersected the whole liver-substance of an infected specimen. They were found only in *Physopsis africana* from the Umsindusi River. At present, no light has been thrown upon the life-history of this cercaria, which has the appearance of an avian trematode.

SCHISTOSOME CERCARIAE

Cercariae which answered to the description of the Schistosome group were found in sporocysts from the liver-substance of twenty-three specimens of *Physopsis africana* (15 per cent) from the brick-fields at Durban. They were present in a lesser proportion of specimens of this same mollusc collected from the Umsindusi River. Bilharzia disease is common amongst bathers in both these places. Except for the absence of eye-spots, the cercariae were identical with the eye-spotted form. The long, slender tail was divided into two short, fin-like prongs. There was no pharynx to be seen. In the *Medical Journal of South Africa* for April, 1916, Dr. J. G. Becker reported that these distome cercariae occurred in *Physopsis africana* from a pool at Nijstroom in the Transvaal. I have seen the microscopic preparations he has made of them. He injected some hypodermically into a guinea-pig and, as I announced at a meeting of the Witwatersrand Branch of the British Medical Association two months later, succeeded in producing three adult male Bilharzia worms in the portal system. This confirmed the opinion that these cercariae, which have only been found in areas known to be infected with Bilharzia disease, are in reality the larval form of the Bilharzia parasite of man.

On April 28, I added some water containing miracida obtained from the urine of a patient suffering from Bilharziasis to a vessel of water containing specimens of *Physopsis africana* from the Umsindusi River. At the end of a fortnight a small sporocyst containing undefined cercariae was seen throwing out branches throughout the liver-substance of one specimen, giving it a yellowish-white appearance. In another specimen a similar young sporocyst occurred; in this could be seen undeveloped cercariae with bifid tails. By the end of three weeks

fourteen out of thirty-one specimens, or 45 per cent, harbored *Bilharzia cercariae*, while only 15 per cent of specimens obtained direct from the river were found to be infected at that period of the year. In another series of experiments, the addition of miracidia to the water in which specimens of *Physopsis africana* were kept, apparently increased the number of infected forms from 22 to 37 per cent, and from 23 to 27 per cent. Similar experiments with specimens of *Planorbis pfeifferi* and *Limnaea natalensis* proved entirely negative.

SNAILS HARBORING "TADPOLE" CERCARIAE, 1916

Date	Source	Species	No. Infected	Percentage
April.....	Umsindusi.....	<i>Limnaea natalensis</i>	2 out of 88	1.6
May.....	(Pietermaritzburg) ..	<i>Limnaea natalensis</i>	0 out of 30	
July.....	(Pietermaritzburg) ..	<i>Limnaea natalensis</i>	0 out of 6	
April.....	(Pietermaritzburg) ..	<i>Physopsis africana</i>	12 out of 197	4
May.....	(Pietermaritzburg) ..	<i>Physopsis africana</i>	4 out of 200	
July.....	(Pietermaritzburg) ..	<i>Physopsis africana</i>	0 out of 6	
May.....	Toll Gate.....	<i>Limnaea natalensis</i>	7 out of 47	13.75
June.....	(Durban).....	<i>Limnaea natalensis</i>	1 out of 12	
July.....	(Durban).....	<i>Limnaea natalensis</i>	3 out of 21	
April.....	(Durban).....	<i>Physopsis africana</i>	1 out of 7	5
May.....	(Durban).....	<i>Physopsis africana</i>	7 out of 85	
June.....	(Durban).....	<i>Physopsis africana</i>	0 out of 13	
July.....	(Durban).....	<i>Physopsis africana</i>	4 out of 131	
April.....	(Durban).....	<i>Planorbis pfeifferi</i>	7 out of 24	30
May.....	(Durban).....	<i>Planorbis pfeifferi</i>	49 out of 163	
June.....	(Durban).....	<i>Planorbis pfeifferi</i>	7 out of 20	
July.....	(Durban).....	<i>Planorbis pfeifferi</i>	3 out of 13	
June.....	(Durban).....	<i>Isadora tropica</i>	0 out of 56	0
May.....	Umgeni (Durban)....	<i>Planorbis pfeifferi</i>	5 out of 15	13.33
June.....	Boshoff St.....	<i>Physopsis africana</i>	0 out of 20	0
July.....	(Pietermaritzburg) ..	<i>Physopsis africana</i>	0 out of 6	
June.....	(Pietermaritzburg) ..	<i>Ancylus</i>	0 out of 20	0
March.....	(Pietermaritzburg) ..	<i>Isidora forskali</i>	0 out of 2	0
April.....	(Pietermaritzburg) ..	<i>Isidora forskali</i>	0 out of 1	

PHYSOPSIS HARBORING BILHARZIA CERCARIAE, 1916

Month	Source	Number	Percentage
April.....	Toll Gate.....	1 out of 7	10
May.....	(Durban).....	13 out of 85	
June.....	(Durban).....	2 out of 13	
July.....	(Durban).....	8 out of 131	
April.....	Umsindusi.....	30 out of 197	18.6
May.....	(Pietermaritzburg) ..	38 out of 170	
June.....	(Pietermaritzburg) ..	7 out of 30	
July.....	(Pietermaritzburg) ..	0 out of 6	
June.....	Boshoff St. Pool.....	0 out of 20	0
July.....	(Pietermaritzburg) ..	0 out of 6	

With the exception of the Schistosome cercariae, we are at present entirely ignorant of the life-history of the various South African cercariae. Some of the "tadpole" forms may give rise to the flukes which occur in sheep and cattle in certain parts of the Transvaal, Natal, and Griqualand East. Others may produce the flukes which I am told are common in the lungs of frogs from some of the pools and rivers of Natal; but, as stated in a letter from Sir Arnold Theiler of the Agricultural Department, "Nobody has yet undertaken to work out the life-history of these flukes in South Africa, and the only reference is that given by Doctor Gilchrist in his book on South African Zoology."

The importance of this study is emphasized by our need of a drug to destroy the adult forms of cercariae in the human host. Perhaps a drug which would destroy the liver-fluke in sheep would be equally efficacious in destroying the Schistosome parasite of man.

NOTE ON A SPECIES OF NOSEMA INFECTING *ATTACUS CYNTHIA* DRURY

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While working on dead larvae of *Attacus cynthia* Drury I met with many individuals which were infected by a species of *Nosema* with spores characterized by shape and refraction of light sufficiently different from those of the silk-worm parasite, *Nosema bombycis* Nägeli, to distinguish it from that species, although more closely allied to it than to any other species of the genus.

The following note is meant to give a short description of the structure of the spores thus far observed, the details of the same as well as the life-history of the parasite being reserved for further investigations.

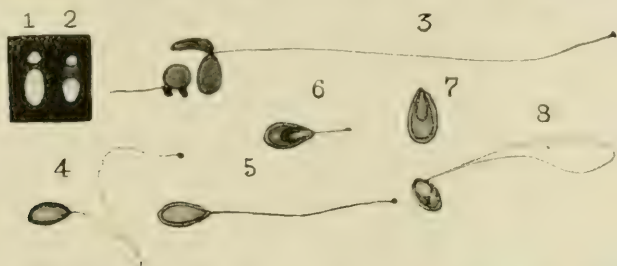
The spores taken directly from the body of the worm were fixed in sublimate-alcohol and stained with Giemsa's solution. Heidenhain's and Delafield's hematoxylin were also used. For the protrusion of the polar filament I employed the method which Kudo (1913) tried with success for the spores of *Nosema bombycis* Nägeli.

The spores (Figs. 1 and 2) are ovoid, tapering toward each end. The refraction of light under the microscope is not so sharp as those of other species. On account of the taper they look long and narrow, like the spores of the species infecting *Anthaerae pernyi* Guér. and *A. yamamai* Guér., but measurements show that this is not the case, the length and the breadth of the spores of the present species being 3 to 3.5 μ and 2 μ , respectively, like those of *Nosema bombycis*, the measurements of which given by Kudo (1916), were 3 to 4 μ in length and 1.5 to 2 μ in breadth, by Stempell (1909) 4 μ in length and 2 μ in breadth, and by Omori (1912) 2 to 4 μ in length and 1 to 2 μ in breadth.

The spore is covered by a thick membrane of a transparent and homogeneous substance like that of *N. bombycis*, but as mentioned above, the refraction of light is not so sharp as in that species. The inner membrane, observed for the first time by Kudo (1916), also exists in the present species and can be pressed out easily. The outer membrane, however, appears to be rather brittle, as it is liable to be crushed into two or more pieces during the process of pressing. With an India ink preparation (Burri's method) the protoplasm stains slightly black, as does that of the silk-worm *Nosema* (Figs. 1 and 2).

The polar filament can be easily extruded from the end of the spore by the method used by Kudo (1913) for *Nosema bombycis*. It is somewhat shorter and thicker than that of the latter, being about 30 to 35 μ in length. The filament ends always in a round knob of special

form (Figs. 3, 4, 5 and 6), which is most probably of a sticky nature. In some specimens of *N. bombycis*, the polar filament has a round end, but not in all, and this again is not so well pronounced as in the present species. Moreover, the filament is not coiled transversely within the spore as in that of *N. bombycis* or of *N. anomalum* Monz., as is assumed to be the case by Stempell in these species. Careful observation of the spore under a high power shows a clear line running longitudinally within the body, which in some appears to be placed in parallels (Fig. 7). These lines undoubtedly represent the filament coiled up within the polar capsule; and when the filament begins to uncoil and a part of it protrudes from the body of the spore, concentric



(All the figures are drawn with the help of an Abbé-Zeiss camera.)

Figs. 1 and 2.—Spores with protoplasm stained slightly black. India ink preparation. In Figure 1 narrow and Figure 2 broad. $\times 1545$.

Figs. 3 and 4.—Spores with extruded polar filaments. The end of the filament has a round point. $\times 1545$. (Figure 3 shows some crushed pieces of outer membrane which are deeply stained.)

Figs. 5, 6, and 7.—Spores containing coiled filament. In Figures 5 and 6 filament partially extruded. $\times 1545$.

Fig. 8.—Polar filament extruded from side of spore. $\times 1545$.

lines can often be seen within the latter (Fig. 6). Sometimes the filament extruded from the side of the spore takes the form of a ring, which is most probably caused by the sticking of the round end of the spore at the base of the filament (Fig. 8).

Finally, I wish to express my hearty thanks to Prof. Dr. C. Ishikawa for his kind advice in the preparation of this paper.

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NOTES ON *POROCEPHALUS GLOBICEPHALUS*

THESLE T. JOB AND A. R. COOPER

Mary L. Hett of the London Zoological Society described and named *Porocephalus globicephalus* from a single mature female specimen, procured from the lung of an American specimen of "moccasin," *Tropidonotus fasciatus* (Linn.). In the Proceedings of the Zoological Society of London, 1915, pages 115-121, she gives the following characters: length, 50 mm.; annulations, about 50; hooks, simple and sharply curved; mouth, pear-shaped with pointed anterior end; head, globular; well marked neck; anus transverse slit on terminal segment.

The above description, which is necessarily meager, is the only reference the writers can find to this species. In view of this condition the following data are herewith reported:

A large black snake, *Basscanion constrictor* (Linn.), was received at the State University of Iowa in the fall of 1916 from Garrison-on-Hudson, New York. When the specimen was killed five males and five females of *P. globicephalus* Hett were found in the respiratory tract. Three males and three females were taken from the lung and two males and two females from the dorsal body wall of the air sac.

The females were found with the head only embedded in the lung tissue, or (those in the air sac) in the musculature of the body, where a copious hemorrhage had been formed. The rest of the body of the parasites was free from attachments, hanging limply in the lumen of the lung or air sac. The heads of the males were not embedded in the tissues of the host, but only superficially attached to the walls of the lung or air sac by the hooks.

The females vary from 82 to 96 mm. in length, being somewhat larger than the specimen described by Hett, while the males were from 14 to 30 mm. long. The color of the female is lemon yellow, the body wall being transparent, thus permitting easy observation of the mass of embryos and the movement of the intestine within. The male is pale cream in color and the body wall is opaque.

The head is globose dorsally; ventrally it is slightly concave with four sharply curved hooks at the anterior edge of the concavity, two on either side of the pear-shaped mouth. The neck is markedly constricted; the body subcylindrical, slightly tapering to the posterior end which is blunt; the digestive tract is seen from the dorsal side; laterally an opaque band runs the full length of the body (this becomes transparent in specimens preserved in alcohol, while the rest of the body becomes opaque). There are about 50 annulations, 48 to 52 having been counted. The digestive tract, which is gorged with blood, is readily seen in the living specimen, and may be traced in preserved ones.

BOOK REVIEWS

THE ANIMAL PARASITES OF MAN. H. B. Fantham, J. W. W. Stephens, F. V. Theobald. New York: William Wood and Company, 1916. xxxii + 901 pages. 423 figures. \$12.00.

As the title page indicates, the work is adapted from the fourth German edition of Braun's well known treatise. It appeared simultaneously in London and New York just after the publication of the fifth German edition of Braun which was reviewed in the *JOURNAL* for June, 1916. A comparison of the views expressed in this work with those in the fifth edition of Braun is especially interesting as it shows the conclusions reached entirely independently by two groups of writers in the same field. It is not surprising that each book treats with greater fulness the work done by investigators of the same nation as the authors, and passes over more briefly the results achieved by workers in foreign nations.

The plan of the work is ideal: the section on Protozoa was entrusted to H. B. Fantham, that on worms to J. W. W. Stephens, and that on the Arthropoda to F. V. Theobald. At the present day the work accomplished in each of these fields is so great and the questions under discussion so involved that no one man can cover them all with equal proficiency. Under the plan adopted here each field is assigned to an investigator who is qualified to write with ultimate authority on the problems in that field, and it would have been difficult to select three men anywhere who would measure up to the ideal better than those chosen.

On the other hand, the time was not particularly propitious for a great work. Other things are in the air that make insistent demands on the attention of all men. There is no leisure for reflection, and concentration on a scientific problem must be well nigh impossible for a man working anywhere in Europe. In truth the book itself shows some evidence of present conditions in the world. It contains a wealth of information on little known topics. It has been brought thoroly up-to-date, even to the extent of two appendices including important material of later date than the general text, and further in having very recent items incorporated on slips bound in between the finished pages at the last moment. This makes the work appear confused, and even in the text there are places where the same impression is given the reader. It seems as if the authors had been working under pressure and the finish one expects in a masterful production had been marred. The volume of scientific material presented to the worker is large and in every way equal to one's expectations, but it is not equally well assimilated at all points. The practice of adding paragraphs here and there incorporates new material at the expense of fluency and the text is not always easily read and understood.

The bibliography is very extensive, covering some pages in fine type, but it lacks all recent items, being in fact but a reprint of the lists in the 1908 edition of Braun. Some references to the literature of the recent items in the text are given in footnotes; but in too many places the new facts are recorded without exact credit, or sometimes without even the name of the author, and the student is left to hunt for himself the precise source of the information. This is least noticeable in the section on Protozoa where footnote references are particularly abundant. It is curious to note that even with such a large space devoted to bibliography one cannot find references to important recent papers by Leiper and other English workers; the authors have treated everyone impartially as not only other references are wanting but even some important papers of Stephens himself are not listed.

The illustrations are numerous and well distributed. They include fewer relics of the past than one usually finds in so comprehensive a work. Most

of the figures are well done and satisfactorily reproduced. One can not help feeling a little disappointed, however, that some have crept in which are new and yet unfortunately inferior. The diagrammatic representations of the *Echinococcus* cysts on pages 352 and 353 are not well drawn and their reproduction on so large a scale is still more open to criticism. A reduction to one-half or one-third the present size would have made their sketchiness less conspicuous without the loss of any important details. In the opinion of the reviewer English and American works are distinctly inferior to continental publications in the character of their illustrations, and the present volume is undoubtedly less deserving of criticism in this respect than most of our works.

Despite its evident minor imperfections this treatise is a valuable and usable work. No one can question that the splendid volume is easily the largest and most complete work on the subject which has appeared in the English language. The work of the printer has been well done and deserves especial commendation. Both paper and type are such as contribute to ease of reading and one lays the volume aside with the conviction that its authors and publishers alike deserve the thanks of scientific workers for the results they have achieved.

JAPANESE MEDICAL LITERATURE, a review of current periodicals the initial number of which was noted in the *Journal* (3:42), has completed its first volume, July to December, 1916. The General Index is well prepared and will be a real convenience even though complicated by the fact that it is paged after the *China Medical Journal* from which the reviews are reprinted, and not according to the reprints themselves. References to animal parasites are numerous and important. The value of the Japanese literature and its great inaccessibility make such reviews of unusual service and American investigators are deeply indebted to Dr. Mills and his colleagues for their work.

The first number of the second volume has also come to hand, and scientific workers gladly look forward to the continuance of this very valuable serial which is without any competition in this difficult and important field.

NOTES

"ECHINORHYNCHUS MONILIFORMIS" IN NORTH AMERICA *

Among the Acanthocephala which are exclusively parasitic and highly specialized for the parasitic habit, only three species have been reported for the human host and even these are rare or doubtful. *Gigantorhynchus hirudinaceus* (= *G. gigas*) is said to occur in man in southern Russia but the statement is unconfirmed. Lambl found in the intestine of a boy a single parasite to which he gave the name of *Echinorhynchus hominis*.

The third species, originally named *Echinorhynchus moniliformis*, has commanded especial attention by virtue of its relation to man. Grassi and Calandruccio found it in Sicily in the small intestine of field mice, rats, and marmots. They detected the intermediate host in *Blaps mucronata* and in some cases found as many as 100 larvae encysted in a single cockroach. They fed such larvae to a white rat and Calandruccio swallowed some at the same time. These developed well in both hosts. The authors identified eggs apparently of the same species in the feces of a young peasant but were unable to carry out a cure and confirm the diagnosis.

Through the courtesy of Mr. G. E. Clark some material has been placed in my hands which belongs to a larger species of closely related type. These worms were taken from a squirrel in Illinois. They furnish the first record of this type for the North American continent. As noted above the European species has been grown experimentally in the human host and this species is likely to show the same power if the mature larvae are introduced by any chance into the human intestine.

After extended study it may be said that the two species are both sufficiently characteristic in their resemblances to each other and in their differences from other known forms to constitute a new genus to which the name of *Hormorhynchus* may be given. *H. moniliformis* (Bremser 1819) is designated as type and attention is called to the fact that Lühe believes there are several species in Europe all included under the one name. The American species is designated as *H. clarki*. Specimens measure 100 to 130 mm. in length. The proboscis is very small, being only 0.255 mm. long by 0.12 mm. broad. The first ring is 5 mm. from the anterior tip. The rings begin faintly but distinctly; they are about 1 mm. long and little wider than the body. They increase rapidly up to 2 mm. in length and at the point of greatest length the individual rings are so swollen as to become markedly wider than the body. From this region they taper out very gradually. The last 15 mm. of the body shows no trace of rings and for the same distance anterior to it the rings are very faint. A full description of the species will be published elsewhere.

HENRY B. WARD

DIPTERA IN THE HUMAN INTESTINE

On Sept. 30, 1916, Dr. W. C. King of Helena, Ark., sent some intestinal parasitic worms which he reported as coming from an adult woman, to the State Hygienic Laboratory connected with the Medical Department of the University of Arkansas for identification. Dr. A. C. Shipp, in charge of the Hygienic Laboratory, consulted with me about these worms. They were plainly annular with about thirteen segments. The shape was very nearly pyramidal with some seven or more papillae at the blunt posterior end.

* Contributions from the Zoological Laboratory of the University of Illinois, No. 91.

These worms looked so much like Dipterous larvae that I suggested that we place them on nutrient agar for a few days to see if they would pupate. Pupation took place in about three days and after about five days more there was hatched a medium-sized black fly. Later others were hatched. Some of these were sent to Prof. James S. Hine of the Entomology Department of Ohio State University, who identified them as *Sarcophaga assidua* Walker.

Our attention has been called to two or three other cases of similar occurrence of what seemed to be Dipterous larvae in the stools of persons this past fall and summer. In one case the sputum gathered in a sterile bottle under a physician's guidance showed what seemed on examination to be young Dipterous larvae. Unfortunately these larvae were killed in the overheating of our incubator.

How these larvae gain access to the intestine unless through ingestion of food in the stage of the freshly laid eggs on exposed cooked food or in uncooked food, is a question. Whether they passed the gastric digestion in the stage of egg or larva is problematical.

CHARLES BROOKOVER

Department of Anatomy, University of Arkansas.

Dr. E. González reported to the Congress of Medicine at Maracaibo the occurrence of *Leishmania brasiliensis* Vianna in a servant woman brought to the clinic at San Fernando de Apure. The preparations were examined by members of the Yellow Fever Commission of the Rockefeller Institute and the diagnosis confirmed. This is the first case of cutaneous Leishmaniasis from Venezuela.

Parasitologists will be interested in the circular of the Merchants Association of New York on the Dangerous House Fly. It is important to urge on every community the necessity of early action this year to eradicate this dangerous agency in the transmission of disease. Incidentally also laboratory teachers may be glad to know that in the flies which appear early in the season a flagellate (*Crithidia* sp.?) occurs abundantly in the gut. Material can be secured readily from this host that is well adapted to class study or demonstration of this group.

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ENDAMOEBA BUCCALIS

I. ITS MULTIPLICATION AND PERIODICITY

NADINE NOWLIN

University of Kansas

Work on these much-discussed parasites was begun with a two-fold object: (1) to find the cause of their periodic disappearance from the human mouth, this phenomenon pointing to a solution of the life-history which has not been solved; (2) to determine by tonsil smears and sections whether this endamoeba is ever intracellular.

MATERIAL

The specimens used in this study were collected entirely from one host and from one point of infection, an upper premolar tooth which was the single focus of infection for a long while. Daily record was kept of the occurrence of these parasites for a period of over five months, from May 1 to July 29, 1915, and from October 10 to December 12, 1916, as well as of health conditions of the host.

Moreover, a study of the parasite was made at various times of day and night with a view to determining whether behavior varies with these conditions.

My attention was first called to the suspected periodic disappearance of *Endamoeba buccalis* by one of my students, who was working on this parasite in the zoological laboratory of the University of Kansas during the winter of 1914. While the supply of material was usually generous, at times she reported it too scarce for study. Since then other protozoology students have reported finding it at times and being unable to do so at others. This did not seem to be wholly due to mouth cleaning, and suggested to me the possibility of migrations of the parasites into the gums or periosteum at the so-called "scarce times."

While I set out with this as a pure hypothesis, my observations during five months have convinced me that *E. buccalis* appears and disappears, tho with not so much regularity as I had at first thought; my journal shows nine scarce times in five months.

Since I have no definite proof for the ideas I am about to set forth in explanation of these migrations, I offer them only as strong circumstantial evidence, with the hope that the suggestion may serve as a good working clue.

METHODS OF STUDY

For diagnostic purposes the fresh smear containing the living material is best. A small amount of scrapings from the point where the tooth joins the gum was spread on a cover-slip slightly heated. This was then placed upon a slide containing a small drop of normal salt solution. If a prolonged study is desired, the slip can be ringed with vaselin before being placed on the slide. I found this method much better than smearing the slide and then covering with normal salt and cover-slip, or than mixing the scrapings freely with the salt solution. The natural environment is most nearly reproduced by the hanging drop method, and it may be due to this that I was able to observe behavior which, so far as I know, has not been reported for this amoeba.

Among *intra vitam* stains, neutral red differentiates the food vacuoles very quickly, but has the disadvantage of staining the whole parasite intensely in a short time. It brings out few points which the unstained, living specimen does not show. After a little experience, there is no danger of confusing the unstained endamoeba on a slide with the leukocytes. The endamoebae have a greenish refractive look which differentiates them even under the low power, where they appear smaller than a pin head. Even motion is not necessary.

For nuclear studies, further investigation of food bodies, structure of cytoplasm, etc., material was preserved, stained according to various methods of protozoa technic and compared carefully for results. Needless to say, the wet film method was adhered to thruout except in one process, and that at the very end of the Giemsa stain, just before mounting in balsam. Comparasion of a slide thus treated with one run into xylol and not allowed to dry, showed no ill effects, and the stain was better without the xylol treatment.

The three methods generally employed were:

(1) The short Giemsa. Bring the wet smear into half and half methyl and ether for 5 minutes. Transfer to a solution of Giemsa made by adding 1 cc. of stock solution to 15 cc. distilled H₂O for 8 to 10 minutes. Wash with distilled water until pink appears. Barely dry in air and mount in balsam.

This gives a beautiful differential stain for the amoebae, coloring the cytoplasm pale blue, their food vacuoles wine, and the nucleus vivid red. This stain brings out a halo of chromidia around the nucleus which other stains do not. Giemsa also differentiates leukocytes clearly

from even the smallest endamoeba because leukocyte cytoplasm stains pink and their nuclei deep lilac. Epithelial cells take a faint pink in the cytoplasm, and a deep pink nuclear stain.

(2) Fixation with the methyl-ether mixture compares favorably with the picro-mercuric-formalin method, which is recognized as one of the most satisfactory fixations for protozoa. This latter fluid used hot and allowed to stand on the smear until it has evaporated almost completely, then washed in 70 per cent alcohol and stained by Dobell's quick hematein method, gives a very clear image of the nucleus, if properly differentiated.

(3) A very similar effect is obtained by hot Schaudinn's fluid (80 parts $Mg\ Cl_2$ plus 20 parts absolute alcohol) followed by Heidenhain's iron-hematoxylin used unripened.

Mallory's stain recommended by Craig (1911) for sections was used on tonsil sections suspected of containing endamoebae, as well as on fresh smears of *Endamoeba buccalis* without satisfactory results.

REPRODUCTION

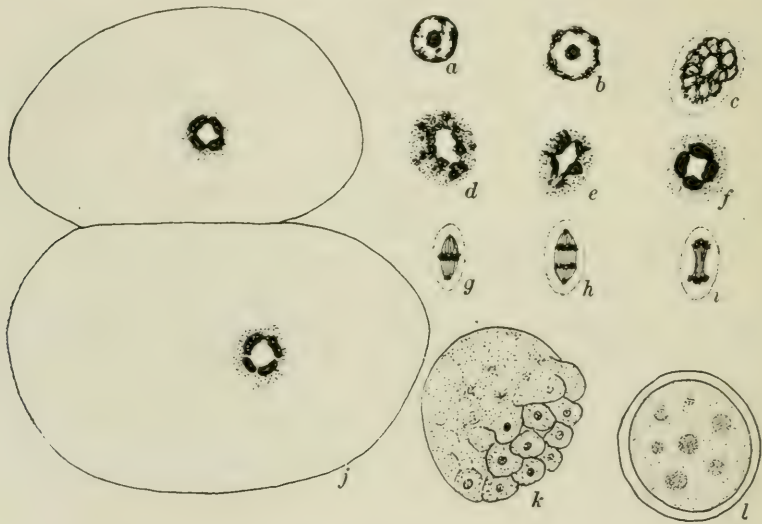
Most of the endamoebae found in smears contain nuclei in the resting stage; and this as pictured in most texts and as shown also in my own slides by the mercurio-picric-formalin fixation and either Dobell or Heidenhain's iron-hematoxylin stains, has a nuclear diameter scarcely one eighth the diameter of the amoeba, a well defined membrane, with its inner margin lined with chromatin granules, and a central body or nucleolus (Fig. *a*). A clear area surrounds the nucleus in fixed material, and since it has been impossible to study it in the living condition, it cannot be determined whether this area is due to shrinkage or is what shows as a pink halo with the Giemsa stain (Figs. *e* and *j*).

Material stained with Giemsa shows outside of this so-called nuclear membrane, and in about the region of the clear area mentioned above, a halo of rose-colored granules, which I interpret as chromidia. Every nucleus stained thus shows this, and I conclude that it is present even in the very earliest resting stage of all *Endamoeba buccalis*, but is not brought out by certain stains.

Craig (1916) believes *E. buccalis* has a primitive type of mitosis. My observations point toward a complex one. During the early stages of mitosis the nucleus enlarges and the karyosome disappears. Chromatin collects on the nuclear membrane, making an irregular border (Fig. *c*) and leaving a clear central vacuole. At times the chromatin of this phase is so organized that it gives the appearance of a spireme, except that the vacuole at the center is always present. A halo of chromidia surrounds the dense chromatin and no doubt contributes to

its formation, since it grows paler as the chromatin condenses. The chromatin condenses into four very distinct bodies and the nucleus remains in this condition longer than in others, judging by the large number found on a slide in the prophase of mitosis.

Whether these four bodies can be compared with chromosomes of higher animals, I do not know, because as soon as the spindle forms they mass and become indistinguishable. Yet they indicate very definite organization during at least part of mitosis. Figure *g* is the typical metaphase, Figure *h* the anaphase, and Figure *i* the telophase. In comparison with early stages of the nucleus these later spindle stages seem very small, the difference being due to the chromidia outside what is usually designated as the nuclear membrane. I doubt now whether



Endamoeba buccalis. For details see text.

a nucleus as represented in Figure *a* is the entire chromatin mass, since I have found chromidia in those of about the same phase stained with Giemsa (Fig. *b*).

The spindles are distinct and give the appearance of having a centrosome at the poles. The size of the nucleus varies considerably, the larger ones being lodged usually in the larger amoebae; but there are frequent exceptions to this (Fig. *j*).

I have occasionally found two equal-sized endamoebae lying in close contact and suggesting binary fission. A close study of these, however, has never revealed a dividing nucleus, but two well formed and widely separated ones. The cytoplasm was in every case completely separated. Very frequently two living endamoebae are seen gliding over each other and becoming so nearly fused that at times the most careful

observations cannot distinguish two separate animals. I have suspected that there may be an interchange of materials between two such individuals, but I have never been able to confirm Craig (1916), who says he saw streaming of cytoplasm from one to the other.

The incessant gliding of the amoebae over each other gives this appearance. This may be conjugation. I have never seen *buccalis* in the actual process of division.

I have never seen buds form and become separated from the parent cell and then develop, tho endamoebae giving the appearance of budding are frequent. At such times the cell resembles the pearl-stage of gregarines, but long enough observation has usually seen them withdrawn. It is usually an adverse condition, such as drying-up or low temperature, which causes this appearance. When proper conditions are restored, normality of form may be resumed.

I have seen about half a dozen stained specimens which I interpret as multiple fission. This is a small number out of the hundreds of specimens passed in review, and yet multiple fission is probably a rare process, which does not take place in the mouth cavity. The suspected forms have no protective walls, have usually been found in close contact with leukocytes or epithelial cells, and are somewhat irregular in outline (Fig. *k*).

There is no limited number of merozoites as eight or four in the *E. coli* and *E. histolytica* cysts, but the number may vary from eight or nine to more than a dozen. Those I have seen do not seem comparable to the reproductive cysts of *E. coli* and *E. histolytica*, but suggest rather the merozoite formation in Plasmodium, and probably serve merely to spread the infection in the host.

Endamoeba buccalis follows the course of all protozoa in encysting. It first becomes spherical and inactive and begins to diminish in size. I induced a kind of encystment once by leaving *E. buccalis* sealed on a slide for six hours with the temperature gradually going down. These did not in that time change much in size, but the food vacuoles paled and seemed to dissolve in the cytoplasm.

Normally, encysted forms are from one-half to two-thirds the size of the active trophozoite; they usually show some faint, rounded inclusions, probably the remains of food vacuoles, and a clear wall slightly spaced from the animal protoplasm proper (Fig. *l*). Encystment seems to be for protection against adverse conditions rather than for multiplication, as is shown by the following observations.

RELATION OF HOST AND PARASITE

Encystment with this form is not as rare as Craig (1916) believes. One strain followed daily for months will show the encysted condition from time to time, and my records show that encystment in *Endamoeba*

buccalis is closely connected with the "scarce periods." As stated previously, daily examinations showed numerous active endamoeba in the scrapings from a tooth for ten and fourteen consecutive days. Then would intervene two to four days when few could be found, though the same region was carefully explored. Such forms as may be found at these scarce times are recorded as "sluggish," "spherical," "encysted." Most of the active forms left were small. Very deep probings into the gums around the tooth sometimes procured a few larger ones, tho finally even these would fail.

Often when the parasites became numerous again they were sluggish and half encysted (Craig's precystic stage) for a day; then they became active and flourished again as usual for two or more weeks. Once they did not disappear for four consecutive weeks, but at the end of that time they were gone completely.

Now what is the explanation of this periodic appearance and disappearance? I laid it at first to mouth-cleaning, until that was carefully tested out. I then considered that changes in the host might account for it, and following out that clue I found by my journal that practically every period of scarcity was accompanied by a time of low vitality on the part of the host as manifested by some slight indisposition. Indigestion accompanied at least three of these disappearances. I concluded that physiological changes of the body, whether normal or abnormal to the host, change the chemical reaction of the body fluids, notably the saliva in this case. These changes may be too slight even to be analyzed, and yet they affect the very sensitive endamoeba living in that medium. As in the case of free-living amoebae, encystment follows when conditions change from the normal, so with *buccalis*, when the saliva changes in its chemical qualities, the parasite encysts either partially or wholly. It withdraws into the periosteum, however, for this purpose.

Further evidence for this was furnished by extracting the infected tooth during a scarce period. The endamoebae had flourished for four weeks and then disappeared; for two days none had been found. The tooth showed an ulcer at the root, and by aid of the binocular many lesions as described by Bass and Johns (1915) were seen on the periosteum. These lesions examined microscopically showed many leukocytes and few amoebae, but on the periosteum around them, and especially where it joined the tooth, amoebae were extremely numerous. These seemed to be in very close contact with the tissue, tho some were removable by means of a fine scalpel. All of the endamoebae thus found were in partial or complete encystment.

I think this withdrawal of *Endamoeba buccalis* to the gums to encyst explains why encystment has been so seldom reported. I believe further that the end of the reproductive phase, binary fission, occurs

normally either in the gum tissue or in close contact with it; for although spindles are frequently seen in smear preparations, actual division of the cytoplasm has never been seen. Since the gums of infected patients cannot be sectioned, I now have under way a study of the tonsils in the hope of throwing light on the intracellular phase of the life-history. An understanding of the periods of disappearance of *E. buccalis* would be valuable in the treatment of pyorrhea if it be caused by this parasite.

TONSIL STUDIES

With a view to determining whether *Endamoeba buccalis* is ever a tissue dweller, I have studied the surface scraping of tonsils both before removal from the patient and after. In only one case did I find endamoeba in the fresh smear, and their detection is certain if they occur. In no case have I been able to identify parasites in the paraffin sections. In view of the fact that Smith, Middleton, and Barrett (1914) found amoebae so plentifully on the tonsils examined, I have been surprised to find none in any of my examinations. If *E. buccalis* is a tonsil parasite, it seems very probable that it penetrates the tissue of so soft an organ and undergoes some interesting stages of development which I believe are constantly taking place in the gums, but which I have no means of demonstrating.

As my work in this direction is scarcely begun, the negative results thus far are by no means conclusive or even discouraging.

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ON THE SPOROZOON PARASITES OF THE FISHES OF WOODS HOLE AND VICINITY

II. ADDITIONAL OBSERVATIONS UPON MYXOBOLUS MUSCULI OF FUNDULUS AND A NEARLY RELATED SPECIES, M. PLEURONECTIDAE OF PSEUDOPLEURONECTES AMERICANUS

C. W. HAHN

Reference to the multiplicative stages of this parasite was made in a former paper (Hahn, 1913). At that time the true parasitic nature of the trophoblasts of both the multiplicative and propagative stages was insufficiently established. The relative virulence of the protozoon and the bacteria also needed further confirmation. Subsequent studies leave no doubt as to either of these points.

In almost every diseased integument, gill, or flesh wound which one examines, there will be found among the decadent tissues a few or many clear, white, even-contoured bodies which rarely take up any stain, no matter what treatment the tissues may be subjected to. The bodies are therefore in strong contrast with the surrounding tissues. If conditions are such that the parasites can be seen at all, the tissues must have taken up more or less of the stain. It was hoped that by using a variety of stains in different combinations with a wide range of fixatives, one might succeed in finding a treatment that would reveal the nucleus and perhaps other cytoplasmic contents of the parasites. No very encouraging results were obtained with the reagents that follow.

After fixation with alcohol (Abs. 62 per cent), ether (32 per cent) and 40 per cent formaldehyd (6 per cent), I used Giemsa, toluidin blue, methylene blue, thionin, Bismarck brown, fuchsin, anilin blue, Bordeaux red, neutral red, dahlia violet, sudan III, indigo carmin, methyl violet, alizarin, rose anilin violet, carbol fuchsin, picro-nigrosin, safranin, and hematein combinations. With corrosive sublimate solutions in different solvents and after chromic, chromosmic, and many other common and some unusual fixatives, such as tannic, malic and formic acids, the following stains were employed: Ehrlich's hematoxylin, Mayer's hematein, safranin, fuchsin, Heidenhain's hematoxylin, and brazilin. Both Mayer's hematein and Heidenhain's hematoxylin give to the cytoplasm of the parasite a slight clouded effect which renders it visible throughout. Rarely a medium or large-sized trophoblast has a faint blue nucleus, and less frequently a small dense spherical nucleus. Brazilin has given promising results when used in connection with a 5 per cent aqueous chromic acid fixation.

The trophic stages of the multiplicative cycle are much more frequently encountered in all the tissues I have examined. They also occur in much larger numbers, especially the minute stages. Thousands of them are frequently distributed more or less equally throughout the myoplasm of certain areas of muscle fibers (Fig. 9). A few are interfibrillar. Such muscle fibers may or may not give evidence of hypertrophy. The size of the parasites in one and the same tissue may vary from 1.5μ to 80 or 90μ in diameter. A very good picture of them has already been published in Figure 12, Plate XX, of the paper mentioned above. The trophic stages of *Chloromyxum clupeidae* (Fig. 8) appear to be very nearly the same in appearance as those of *M. muscoli*.

In shape the multiplicative trophoplasts are circular or oval when small. Older ones have slight blunt extensions here and there over the surface. Occasionally a long pseudopod is encountered. Since these observations are made from fixed smears, it is probable that in life the display of activity on the part of the pseudopods would be very striking, could it be seen. As yet I have observed no striking activity in numerous fresh tissues. In very large parasites the cytoplasm is finely granular. The smaller ones appear to be structureless. Trophoplasts of moderate size frequently have a thin border of stainable material covering a part or all of the surface. This suggests an excretion or surface deposit, but is in reality what remains of the muscle nucleus which has been atrophied under the action of the parasite (Fig. 4). This can be demonstrated by the study of a large number of cases, when it will be found that there is a complete series of stages between the condition here described and normal nuclei.

Multiplicative trophoblasts have been found in muscle epidermis, gill epithelium, and connective tissue. All of these tissues are attacked and undergo cellular degeneration. The nuclei and mucous cells usually remain in various stages of hypertrophy and constitute a very misleading series of artifacts.

The staining reaction and the general appearance of the multiplicative trophoplasts are such as to suggest strongly that these bodies are some fatty or lipid degeneration product. After many months of doubt, preceded by many more during which they were overlooked because it was assumed that the bodies in question were oil globules, it finally proved impossible to exclude them from the myxosporidian life-history. Authority may be found in the literature in support of both interpretations. It is an accepted fact (Adami, 1910) that with the hypertrophy of muscle, uniformly distributed fat bodies are to be expected. It has also been shown that the hypertrophy of the nucleus sets up changes in its immediate vicinity that result in lipid substances.

The association of hypertrophied nuclei and Myxosporidia described above fits these specifications very well. On the other hand, small globular bodies within and between the muscle bundles were taken by Pfeiffer (1891: 106) to be germs of a myxosporidian.

The evidence upon which I have based my decision is (1) the failure of either osmic acid or sudan III to give a fat reaction, whereas oil globules on the same slides give a typical reaction. For the sudan III tests the tissues were fixed in aqueous formaldehyd solution, treated with a low-grade alcoholic solution of sudan III, and preserved in glycerin. (2) The large trophoplasts show granular cytoplasm and a faint nucleus at times, when stained with Mayer's hematein and Heidenhain's hematoxylin. (3) The trophoplasts occur in graded sizes as if belonging to the same stage of growth. (4) Many trophoplasts have pseudopodial extensions that have a strong motile suggestion. (5) Many muscle fibers in an advanced stage of hypertrophy are free from the bodies in question; they have migrated or operated in some other part. The products of degeneration would be expected to be uniformly distributed in all atrophied muscle fibers. (6) The sporoblasts of both *M. musculi* and *Chloromyxum chupeidae* have exactly the same oil-like appearance as the multiplicative trophoplasts and reactions, but contain some characteristic body that belongs to the sporogenesis, such as the myxospore itself (*Chloromyxum*) or the sporoblast nuclei. (7) When one compares the trophic stages of the multiplicative cycle with the propagative cycle of the *Myxobolus* or both with similar stages of the *Chloromyxum*, four kinds of bodies may be recognized. If the structures in question are artifacts, this distinction into two classes would not conform exactly to the conditions required by the protozoon life cycle as to equality of development of all individuals present. This is exactly what is found in regard to both of the genera here described. Either all the parasites are young trophoblasts of the multiplicative cycle, or all are in some phase of the propagative cycle. (8) Many of my preparations have been treated with ether and absolute alcohol. Oils are extracted by this treatment. Yet most of the structures in question show some evidence of a solid content, whereas casts of fat bodies, when encountered, are clear and structureless.

Many observers have found in fresh tissues small motile, structureless bodies, and also cells with nuclei, which they have assumed were parasites. I have examined fresh infected tissues of both the herring and *Fundulus*. While able to recognize the trophoplasts and sporoblasts, it has never been possible to be certain that the suspected objects were parasites until they were either connected by stages to sporocysts containing myxospores or until they had been verified in fixed and stained preparations. Pathological tissues frequently con-

tain artifacts resulting from the products of degeneration (Hahn, 1913:197) which are very misleading. There are also numerous tissue cells and ameboid cells with well-developed pseudopods in atrophied tissue, especially in the epidermis of such fish as the flounder. Under these circumstances, one is inclined to place little confidence in observations based upon fresh tissue alone. It is probable that the observations of Pfeiffer (1893 and 1891), Thélohan (1893), and Megnin on the trophic stages of *M. pfeifferi* were correct, but one must always feel doubtful about the reliability of one's interpretations when good and sufficient reasons for considering any fresh cell as a parasite are not given.

The multiplicative trophoplast continues to grow in size until it is over 50μ in diameter. Although not observed alive, the shapes and general appearance lead to the conclusion that they are motile. They usually occur singly in comparatively uninfected portions of the tissue. In shape they vary from a long gregarine-like structure with very finely granular endoplasm and a shallow clear cytoplasm, to a smooth oval or circular mass when seen in profile. These large individuals may reach a diameter of 50μ (Fig. 14). Associated with them in the same tissues one rarely finds schizonts containing minute spores. The schizonts range in size from 40 to 55μ . These bodies are embedded in the muscle fiber in a cavity which they completely fill, giving precisely the same appearance as the large immature schizonts (Fig. 12). The multiplicative spores within are about 1.5μ in diameter. In the few cases which I have examined, they have not taken up the stain, but are visible owing to the presence of a residual material which retains a moderately intense stain. Free multiplicative spores are common, and like all multiplicative stages, they are also characterized by the non-staining quality. Occasional schizonts containing spores are encountered in fresh tissues. They have also been seen and distinguished from propagative stages in sections. As yet none of the latter were so large as those here figured. The scarcity of sporulating schizonts is no doubt to be attributed to the rapidity of the dissemination of the spores under the muscular activity. As previously noted, the schizonts have already migrated into fresh tissues by the time they have reached any considerable size.

One might suspect that the bodies produced by the so-called schizogony and figured here are bacteria. Very similar colonies of bacilli have been described elsewhere (Hahn, 1913). These bacilli do not occur in muscle which is essentially normal, and they are not accompanied by interstitial material when isolated and embedded in the myoplasm. The individual here figured was fixed with Flemming's fluid and stained with safranin. This combination cannot be expected to stain bacteria. Failure to stain with Giemsa and methylene

blue does occur in certain bacilli which are common in the necrotic region of these sores. The stain is therefore not so reliable a criterion as location, uniformity of size, association with other free individuals, etc.

The time required for one complete multiplicative cycle is approximated in the discussion of inoculation experiments.

Schizogony in *M. musculi* would be expected if the schizonts containing spores had not been seen, since the peculiar distribution of the trophoplasts cannot be readily explained by any other kind of multiplication. The smallest individuals are usually very numerous in localized regions and differ but little in size. Older stages occur in fewer and fewer numbers unaccompanied by the small forms, proving that they have migrated from the focus of the multiplicative process.

Multiplicative reproduction in Myxosporidia was demonstrated by Cohn (1896) in *M. lieberkühni* and by Doflein (1898) in *Glugea lophii*. Minchin (1903) describes fission and budding and refers to the multiplicative schizogony of *Glugea lophii* as a kind of schizogony, adding that "this kind of reproduction is probably very common, if not universal, in the tissue and cell-infecting Myxobolidae and Glugeidae." Doflein (1911) makes provision in an outline of the life-cycle of a typical myxosporidian for schizogony and suggests it is typical as a preliminary to sporogenesis, but gives no specific illustrations and does not elaborate this as a process of multiplicative reproduction independent of the formation of sporoblasts.

Laveran and Mesnil (1902) review the various methods of reproduction in Myxosporidia, referring to budding as described by Cohn in *Myxidium lieberkühni*, also to binary fission as described by Doflein in *Chloromyxum leydigi*, and to the simultaneous division of the nuclei in the process of plasmotomie, but cite no typical cases of schizogony.

The occurrence of multiplicative schizogony in a species of *Myxobolus* in the bile of the flounder has been observed by the writer. Plehn (1905) figures and describes a schizont with a large number of multiplicative spores in *Lentospora cerebralis* from the salmon. It causes the so-called "twist disease" (drehkrankheit). He supposes that the spores develop into a cell which has a conspicuous nucleus that is lacking in the spores. In view of what has been learned about *M. musculi*, it seems more probable that Plehn's nucleated cells are in the line of the propagative cycle. They are probably sporoblasts or gametoblasts. The non-nucleated spores are perhaps multiplicative spores.

A schizont with ten multiplicative spores has been described by Nemeček (1911) in *Henneguya gigantea*.

It is now certain that the propagative cycle starts with a spore which is unlike the meront of the multiplicative stage. Beginning with

the spore, the staining properties of the propagative trophoplast are distinctly different. In the paper already cited, Figure 14, Plate XX, represents a schizont with differentiated spores. They are probably not multiplicative spores as there stated. The latter are smaller and their nuclei do not stain. The propagative spores occur free in the myoplasm in fewer numbers, but with about the same pathological effects and habits as the multiplicative spores. Because of the intimate and constant association of the small propagative spores having nuclei with large ameboid trophoplasts (Fig. 14), I have concluded that the former develop into the latter. This view might be less tenable if there was not a sharp limitation to the range of development which the parasites have attained in any given tissue.

The fate of large propagative trophoplasts such as are shown in Figure 14 is probably some form of multiplication which results in moderate-sized sporoblasts. It is possible that they develop directly into primary sporoblasts, such as are undoubtedly represented in a trophic condition in Figure 5, and in a quiescent condition in Figures 6 and 7. Such an interpretation conforms to the accepted life-history for other species of *Myxobolus*. But if one is right in supposing that certain elongated spores of moderate size which have been occasionally encountered isolated (Hahn, 1913: 113, Figs. 17 and 19, Plate XXI), and in small sporogenic cells (Ibid.: Fig. 16, Plate XX), are to be included in the life cycle of *M. musculi*, then it is difficult to reconcile the stages represented with the sporogenesis as hitherto described by Mercier (1908), Keysselitz (1908), Schröder (1907, 1910), and others. There are apparently three different kinds of spores in *M. musculi*. One belongs to the multiplicative cycle and may without doubt be called an asexual type. The other two are very probably to be associated with the propagative cycle, and one may expect that they have some sexual significance. Of these, one is a spherical spore 2.5 to 3 μ in diameter, which has a small well-defined nucleus and faintly staining protoplasm. They occur in large schizont cysts (Hahn, 1913, Fig. 14, Plate XX), and are produced in rather large numbers. They no doubt become the sporoblasts that are so numerous in tissue adjacent to them in the one tissue where they have been encountered. The latter are identical to sporoblasts such as are figured in this paper (Figs. 5, 6, and 7). The other type of propagative spore was encountered in the same slide as the above and in the immediate vicinity of them. They are contained in cells having a diameter of about 12 μ . These sporocyte cells appear to be of independent origin. They occupy the space left by an atrophied muscle fiber. The contained spores are 2.5 by 4 μ in size and have rather large nuclei. Each sporocyte contains from four to twelve spores.

The elongated type of spore not yet has been satisfactorily explained. If the spherical spores which contain a stainable nucleus are identical with what was assumed to be multiplicative spores, the elongated spores may prove to be sporocytes. I am not altogether certain that the one tissue represented was not harboring a double infection. A third hypothesis is that the small spherical spore is a microgamete and the larger elongated spore is a macrogamete. In this connection it is interesting to note that in the muscle fibers where typical medium-sized sporoblasts are abundant, occur also several small elongated cells with pointed, densely staining nuclei, having a terminal position (Hahn, 1913, Fig. 18, Plate XXI). One may suppose that these are motile microgametes, but at present no evidence is available to substantiate the hypothesis.

One may conclude with reasonable assurance that the sporoblasts do arise from a very common type of spore which arises by a process of schizogony, and that the propagative sporoblasts are sufficiently differentiated from the multiplicative spores to be easily distinguished while yet in the schizont cyst. I believe that after a succession of multiplicative cycles ending in multiplication by schizogony, there follows a schizogony which generates spores that become differentiated very early into either gametes or primary sporoblasts. (See also page 102 in the first section of this paper for time relations.)

The primary generative cells of *M. musculi* certainly do not arise by free cell formation in large myxoplasms, such as is the case in *M. pfeifferi* of the barbel, and *Sphaeromyxa labrazesi* (Lav. and Mesnil), according to Schröder, 1907. The primary propagative cells of *M. musculi*, on the other hand, are set free simultaneously by one or the other of the schizonts described above. This conclusion is based not only upon the existence of two or more types of schizonts, but upon the fact that in four tissues where sporoblast stages occur, they are very numerous and at approximately the same state of development.

The propagative stages have not been encountered so frequently as the multiplicative stages. This is probably due to the fact that they are not nearly so abundant. In some tissues one may find both kinds present, but according to my observations, one or the other is always greatly predominant. With the exception of the elongated spores which occur in certain small cysts that have been figured and described elsewhere (Hahn, 1913: 204, Fig. 16, Plate XX), there is no evidence that the multiplicative and propagative trophoplasts do not have practically the same structure and appearance when small.

There is absolutely no evidence that they are generated consecutively by budding or fission or plasmotomie, but quite the contrary. The propagative stages gradually differentiate from the multiplicative type, and by the time one can positively identify them as such, they are dif-

ferent both in appearance and staining reaction. When unmodified by the contraction of the muscle fibers, they are more or less spherical bodies with almost transparent glassy cytoplasm and a small vaguely staining nucleus (Hahn, 1913). Older conditions are shown in Figure 18 of the paper just referred to. They have a large well-stained nucleus and fit loosely in the space which they have eaten in the myoplasm. The shape varies from round to oval, and evidence of active mobility or of pseudopods is often lacking. Somewhat earlier stages, when compressed by the shortening of the muscle fiber, have long extensions of the cytoplasm (Fig. 5). The nucleus is also extended into a long slender mass and sometimes extends into the thicker portions of the protoplasmic branches. This condition does not seem to be quite normal. Many cases of less compressed myxoplasm occur as regularly distributed spindles.

Besides very small sporoblasts, there are numerous good examples of larger sporoblasts and sporocysts in all stages of sporogenesis and sporocysts with immature and more or less mature myxospores. Stages not figured in the plate of this paper will be found in my paper of 1913.

When unmodified by the contraction of the muscle fibers, the sporoblasts are probably more or less spherical with a small nucleus (Fig. 7), or a large one (Fig. 6), and almost transparent vitreous cytoplasm. The nucleus does not stain intensely, but is more or less free from characteristic stainable bodies (Hahn, 1913, Figs. 18, 21 and 35, Plate XXI). Presumably these are the same stage of the organism as those which are encountered frequently in an ameboid condition fitting loosely into irregular transverse clefts of hypertrophied muscle fibers (Fig. 5). The conditions in some cases, such as Figures 6 and 7 here and Figure 18 (Hahn, 1913), suggest that there is an advanced condition in which ameboid activity is lost. If so it is probably just preceding the process of sporogenesis. There is a transition between the ameboid condition and the inactive condition wherein the myoplasm is divided into narrow transverse partitions by very numerous spindle-shaped cells which lie with their long axis at right angles to the length of the fiber. It is unsafe to say to just what extent the mechanical action of the muscle and the number of parasites are responsible for these alterations in shape. Thélohan (1891) figures and describes exactly the same condition in fish muscle fibers. He also interprets them as sporogenic cells.

Sporoblasts are sometimes so closely packed in the space once occupied by a muscle fiber that, though the form of the fiber remains, the myoplasm can be seen only rarely (Fig. 18). When thus packed together, these cells form a pseudo-epithelium which can be distinguished from a slightly degenerated epidermal or mucous epithelium with the greatest difficulty. Practically one must depend in many

cases upon a general resemblance to other epithelial masses in the same tissue, the cells of which have entered upon some easily recognizable stage of sporogenesis. Such pseudo-tissues are either more or less obscured by the hypertrophied myoplasm, muscle, and vascular nuclei, or are so closely packed that unless spread out mechanically in smear preparations, suitable specimens for drawings cannot be found. It is such a scattered group that was selected for the camera drawings represented in Figures 7 and 18. For purposes of reproduction it was necessary to exaggerate the detail of both nuclei and the cytoplasm of the parasites. The disinclination to stain is still retained to a limited degree in the propagative stages.

The epithelioid tissue just referred to must not be confused with another condition which has already been described (Hahn, 1913), in which the hypertrophied nuclei of vascular and connective tissue occupy the mold of a muscle fiber and, mingled with the remnants of the myoplasm, resemble a bit of degenerating epithelium.

The identity of the cells of which these pseudo-tissues are composed rests upon very positive evidence. Not only can one easily find obvious differences between them and true epithelium, but there are many such masses lying among the atrophied muscle fibers, many of which are in stages of sporogenesis like that represented here (Fig. 3). On a single slide one cannot fail to connect stages identical to those of Figures 3 and 6 (below) with the less obvious stages in Figures 7 and 18. There are also interesting isolated groups of sporoblasts identical in appearance to those forming the epithelioid masses that occupy small spaces in the myoplasm (Fig. 6). Differences in the size of the nuclei are to be expected when it is recalled that we are comparing primary and secondary sporoblasts with pansporoblasts and possibly other stages of the propagative cycle. Figure 7 is magnified 560 diameters and Figure 6, 750 diameters. It is noteworthy that the group in Figure 6 is accompanied in the same fiber by a pansporoblast with ten or eleven nuclei. Between the former and the latter the hypertrophied myoplasm has lost the fibrillae. That the bodies represented in this fragment of muscle fiber were invading parasites is clearly obvious. The muscle hypertrophy alone is significant. Adjacent fibers have numerous isolated parasites, while the epithelioid masses and numerous stages of sporogenesis like Figure 3 are on the same slide from which the group in Figure 6 are taken.

It is rather by analogy with other *Myxobolus* than by direct observation that one must interpret the various propagative stages which have been encountered in the tissues of *Fundulus*. The majority of the older stages such as those in the pseudo-epithelium are probably sporoblasts. As already stated, those with large and small nuclei may possibly be gametoblasts. There are some very large spherical stages

with two large and two small nuclei from which the sporogenesis starts. With numerous succeeding stages leading up to Figure 3 one has, at least, ample proof that trophoblasts whose nuclei stain are destined to give rise to propagative spores, i. e., myxospores.

It is of considerable interest that the early propagative stages like trophoplasts have a destructive career. Their scattered distribution in the younger stages is due to a rather extensive motility either upon the part of the parental schizont or upon their own activity. But when nearly mature they evidently become less active. The masses which occupy the mold of the muscle fibers suggest in a general way pseudocyst formation such as has been found in the gill (Textfigs. 1, 2, and 3), and is common in many of the other species (*M. pfeifferi* of the barbel disease).

The pseudo-epithelium (Fig. 18) formed by the propagative stages of *M. musculi* is a most remarkable condition and deserving of more attention. The simulation of normal or slightly hypertrophied host tissues is a most deceiving circumstance. When a parasite having such qualities occurs in small numbers and more or less isolated, the most careful observer will fail to recognize it. Moreover, if a sufficient number of tissues is not available, suitable stages for a positive identification will be wanting. The facts just noted are important because of their possible bearing upon the epithelioid tissues of mammalian cancer. Adami (1910) states that cancer tissue resembles nothing so much as a parasite upon the mammalian tissues. The propagative stages of *M. musculi* frequently give the appearance of a typical epithelioma.

SUMMARY

For the results of inoculation experiments bearing upon the life-history see the summary at the end of the first section of this paper.

1. *M. musculi* has a series of multiplicative cycles starting with the myxospore, followed by a propagative cycle, ending in the myxospore.

2. There are two or more types of schizonts and schizogony.

3. Multiplicative reproduction is carried out by means of a large schizont which gives rise to a very numerous progeny of very minute spores.

4. The multiplicative spores and trophic stages do not take up any stain thus far utilized, with one not very satisfactory exception.

5. Multiplicative trophoplasts and schizonts migrate into uninfected tissue, particularly just before the quiescent period preceding schizogony.

6. All propagative stages possess a nucleus which reacts to basic stains.

7. The schizonts which give rise to primary propagative spores also migrate into new tissues before undergoing schizogony.

8. Another process of schizogony exists in which the schizont is very large and the spores, though larger than multiplicative spores, are small and have a small nucleus which reacts to a basic stain.

9. A third type of propagative schizogony may possibly exist in which the schizont is small and the spore very large, with a large nucleus which reacts to a basic stain.

10. If the conditions in 8 and 9 are trustworthy, there is a differentiation of gametes into macro- and microspores.

11. Sporoblasts, whether arising from conjugation or destined to conjugate, are ameboid, trophic, having the ability to migrate to a limited extent only when immature, and losing this property later.

12. Multiplicative stages perforate muscle fibers extensively and bring about profound hypertrophy. Propagative stages while yet trophic are also predacious, but to a less degree. The latter give rise to characteristic irregular transverse clefts in the fibers. Such clefts vary in number, shape and size, and occur in more or less atrophied fibers only.

13. The passive propagative sporocytes pass through all the characteristic stages of sporogenesis such as have been described for *M. pfeifferi* (Keysselitz, 1908).

14. Closely packed primary and secondary sporoblasts form an epithelioid tissue which at times has the appearance of integumentary epithelium and closely resembles mammalian epithelioma.

15. Pseudocysts occur, having many myxospores in a common sporocyst plasm. They probably arise by the fusion of closely packed sporocysts.

MYXOBOLUS PLEURONECTIDAE OF WINTER FLOUNDER

A winter flounder (*Pseudopleuronectes americanus*) having open sores was collected by Dr. W. E. Sullivan in the vicinity of Woods Hole. When examined, the flesh proved to have undergone pathological changes almost identical to what has already been described in *Fundulus*. The flounder was 8 inches long and had three lesions. One on the dorsal side was $\frac{3}{4}$ inch wide and 1 inch long; the other two were smaller. The integument was either white and partially decomposed or completely gone. The underlying flesh was red and vascular at the surface and less transparent than normal. These external conditions resemble the appearance of the myxosporidian disease of *Fundulus* as much as one could expect, considering the difference in the integument, skin, and color of the flesh of these fish.

Suitably stained smear preparations of the flesh present almost the same pathological conditions as are found in the fundulus disease.

There are present hypertrophied muscle fibers and epithelium cells, degenerated nuclei, mucus cells, and numerous bacteria limited to the most disintegrated parts. Numerous fibers contain considerable numbers of unmistakable trophic stages of the multiplicative cycle of a *Myxobolus*. One could not distinguish these from the same stages of *M. musculi* of the Fundulus. Large multiplicative schizonts, almost mature sporoblasts, and myxospores are also to be found in the same tissues. With the exception of the myxospores, there is no noticeable difference in the propagative stages and those of *M. musculi*.

The myxospores are not very abundant, but they are suitably stained for comparison with other species. One has no difficulty in distinguishing them from the myxospores of *M. musculi* by their shape (Fig. 2). The latter are tapered more at the polar end and the polar capsules are drawn out into a narrow apex.

The flounder parasite has myxospores which are 14.8μ long and 11.9μ wide. Those of *M. musculi* are 14.3μ long and 6.7μ wide. Immature myxospores of *M. musculi* are 12 by 7.5μ by actual measurement. In both cases the figures here given are the average of several different spores. The flounder myxospore has polar capsules which are 6μ long by 3.7μ thick, and the fundulus parasite has polar capsules 6.5 by 2.0μ . The flounder myxospore is therefore 2μ shorter than *M. pfeifferi* and 1.9μ narrower. In shape and appearance it resembles the latter closely. Allowing for slight variations in size and shape due to difference in maturity, the discrepancy between the myxospores of the two fish is too great to consider them as belonging to the same species. The inoculation of one host species by myxospores from the other will easily settle this question. In the meantime, the name *M. pleuronectidae* is proposed for the flounder parasite. It is probable that many species of the flatfish are subject to attacks by this parasite.

It is interesting to note that one *Chloromyxum* myxospore was encountered in the tissues of this flounder.

The articles cited in this portion of the paper will be listed at the conclusion of the paper in the September number of the JOURNAL.

EXPLANATION OF PLATE

Fig. 1.—Five sporoblasts of *C. clupeiidae* from the same slide as Figure 16. Note the unstained cytoplasm of the sporoblasts with thin filaments of host tissue residues separating one sporoblast from another. Compare this non-staining material with that in Figures 8, 11 and 16. Note the square form of both myxospores and sporoblasts. The capsule nuclei are applied to the polar capsules in the right hand lower sporoblast. The sporoplasm is unstained. The sporoblasts each contain a developing spore the nuclei of which are probably imperfectly stained. $\times 1575$.

Fig. 2.—A myxospore of *M. pleuronectidae* from a lesion of the back of a winter flounder (*Pseudopleuronectes americanus*). $\times 1575$.

Fig. 3.—A sporoblast of *M. muscoli* undergoing sporogenesis. The specimen here represented is one of many in a mass of cells similar to that in Figure 7. The dark border is stained serum. A space exists between the latter and the sporoblast, due to shrinkage. There are about 30 well defined nuclei. A few appear to be elongated as if about to divide. $\times 560$.

Fig. 4.—Three trophoplasts of *M. muscoli* from the eye muscles of an inoculated Fundulus that had died from a general infection of the head region. The parasite has not taken up any stain while the host tissue has. These three cases show as many stages in the hypertrophy of muscle nuclei which the parasites have apparently attacked. Note the small trophoplast is associated with a nucleus showing normal alveoli while the larger trophoplasts are associated with nuclei from which alveoli have partially or completely disappeared. $\times 1575$.

Fig. 5.—A fragment of an atrophied muscle fiber from a large open lesion of Fundulus containing propagative trophoplasts, possibly sporoblasts of *M. muscoli*. I regard these as earlier than in Figure 6. The position of the long axis of the sporoblasts and their cavities, which is at right angles to the length of the muscle fiber, is due most likely to the contraction of the fiber. Compare the granular nuclei in this with Figures 6 and 7. $\times 300$.

Fig. 6.—A muscle fiber from Fundulus with several sporoblasts of *M. muscoli* in the same cavity and one isolated. The many small nuclei of the latter indicate that it is in an advanced stage of sporogenesis. The large size of the nuclei in the others indicates that they are in a much later condition than those shown in Figures 5 and 7. Atrophy of the myoplasm is just beginning. $\times 750$.

Fig. 7.—A group of sporoblasts of *M. muscoli* at about the same stage of development as in Figure 5. The group lies adjacent to an epithelioid tissue which has replaced a completely atrophied muscle fiber. These cells are drawn out of the mass sufficiently to permit drawing details which are not possible in the compact masses, one of which is shown in Figure 18. $\times 560$.

Fig. 8.—Portions of four muscle fibers from the dorso-branchial region (not body muscle) of the young of *Clupea harengus* which had numerous pseudocysts of myxospores of *C. clupeiidae*. No myxospores occur in the head region. All the muscle is thus riddled with the trophoplasts of the *Chloromyxum*. They are both inter- and intra-fibrillar. When intercellular, note they have crowded the muscle fibers. Fibrillation and striation of the muscle fibers is entirely lacking. $\times 300$.

Fig. 9.—A portion of a muscle fiber from the body muscle of *F. heteroclitus* having a typical infection of very young multiplicative trophoplasts of *M. muscoli*. $\times 300$.

Fig. 10.—Three mature myxospores of *C. clupeiidae* showing the four polar capsules stained. $\times 1650$.

Fig. 11.—Sporocyst of *C. clupeiidae* in an atrophied myoplasm from anterodorsal body of muscle of young *C. harengus*. Compare with Figures 1 and 16. Note the increase in size. Sporocyst plasma is unstained. Sporoplasm has assumed a more or less rectangular form. $\times 1575$.

Fig. 12.—A multiplicative schizont of *M. muscoli* in the myoplasm of an atrophied fiber from Fundulus. $\times 750$.

Fig. 13.—A medium sized trophoplast of *C. clupeiidae* migrating from an old tissue to new. $\times 300$.

Fig. 14.—A large schizont of *M. muscoli* from the same slide as Figure 12 which has not yet undergone schizogony. $\times 560$.

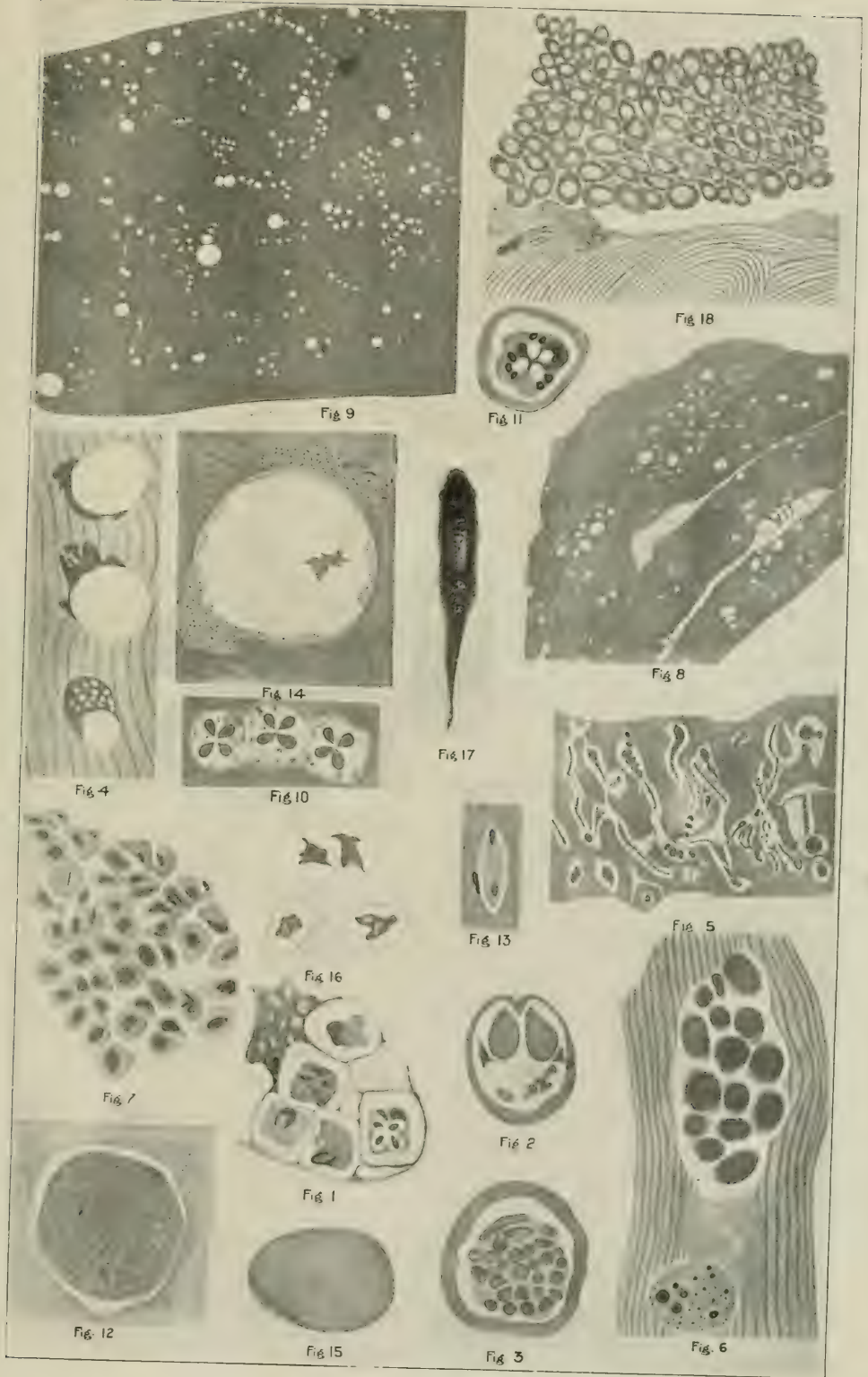
Fig. 15.—A large schizont of *C. clupeiidae* from the body muscle of young *Clupea harengus* in which no pseudocysts are present and no myxospores were found. These schizonts are abundant in comparatively normal muscle fibers. $\times 300$.

Fig. 16.—Four sporoblasts of *C. clupeiidae* from inflamed body muscle in the ventrolateral region. The two left-hand sporoblasts are enclosed in the sporocyst and the right-handed sporoblasts are free. The latter are comparable to the shaded portions in Figures 1 and 10. $\times 1650$.

Fig. 17.—Photograph of a typical lesion in *F. heteroclitus* which afterwards proved to be caused by a typical infection of *M. muscoli*. Note the swelling, the loosened and projecting scales, and the open central area from which the integument has disappeared.

Fig. 18.—A mass of sporoblasts of *M. muscoli* giving the appearance of an epithelium. The truncated form of the mass is due to the fact that these sporoblasts have occupied the space left by the muscle fiber whose hypertrophy they have brought about. $\times 300$.

PLATE



CONTRIBUTIONS TO THE STUDY OF PARASITIC PROTOZOA. II.*

MYXOBOLUS TOYAMAI NOV. SPEC., A NEW MYXOSPORIDIAN PARASITE IN
CYPRINUS CARPIO L.

ROKUSABURO KUDO

While studying Cnidosporidia in some fresh-water fishes during the last few months, my attention was attracted to a minute white spot on the branchial lamella of a *Cyprinus carpio*. Examination under the microscope showed that the white spot was no other than a round cyst of a myxosporidian containing numbers of ripe spores each having only one polar capsule. The fish that harbored the Myxosporidia was a year old, having a length of about 6 cm. On searching carefully all the branchiae of the fish under the dissecting microscope, I found another round body situated near the free end of a branchial lamella, the diameter of which was about 200μ . Since that time, many fishes of the same kind, and reared in the same pond where the above-mentioned infected fish had been found, have been examined for the same parasite, but it has not been found again. Consequently, the material is too scanty for detailed study. I will try, however, in the following pages, to give the results of observations on the one fish, which are probably of some interest, since the morphology, and especially the life-history, of the unicapsulated Myxosporidia seem, so far as I am aware, to have been left in obscurity.

The branchiae of the infected fish were cut into pieces, fixed with Schaudinn's or Fleming's fluid, imbedded in paraffin, cut in serial sections of 2 to 4μ thickness and stained with Giemsa's solution or Heidenhain's iron hematoxylin, the latter being counterstained with eosin or orange G.

MORPHOLOGY OF THE TROPHIC STAGE

I expected that in sections would be found many young developmental phases of the organism which could not be observed externally in the fresh state, but in the study of the numerous sections, to my disappointment, only very few of the parasites were observed, showing that the infection in the present case was one of slight degree. The focus of infection was the connective tissue of the gill filament. The connective tissue became swollen by the infection of the parasite, and with its growth the tissue around it formed a thick layer, penetrated by numbers of capillaries (Figs. 1 to 3). A similar phenomenon has

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already been observed by Cohn in *Myxobolus minutus* and by Schröder in *Henneguya accrinae*. The parasite in the branchiae is generally ovoidal in form (Fig. 1), but sometimes a calabash-shaped one is present (Fig. 2). This is probably caused by the union of two closely neighboring individuals. The gill-filament infected is not so greatly swollen as is the case with *Henneguya acerinae*, *Myxosoma dujardini* according to Thélohan (1895), and *Henneguya gigantea* according to Nemeček.

The youngest form found has an oval shape. The dimensions are about 67 by 50 μ , showing clearly the differentiation of the protoplasm into ectoplasm and endoplasm. The ectoplasm exhibits vertical striations (Fig. 3), similar to those of *Myxidium lieberkühni*, *Myxobolus pfeifferi* according to Thélohan (1895), of *Henneguya acerinae* and also of *Sphaeromyxa sabrazesi* according to Schröder (1907), and *Myxobolus gigas* according to Auerbach. Besides this structure, in some specimens the ectoplasm differentiates very fine plasmic processes, 2 to 3 μ long, from its surface (Fig. 3). Auerbach (1909) noted a structure analogous to the above-mentioned one in *Myxobolus fuhrmanni*, but he could not determine whether it belonged to the parasite or to the tissue of the host. Schröder (1907) observed a similar differentiation of the ectoplasm in *Sphaeromyxa sabrazesi*, stating that "an der Oberfläche des Ektoplasms erkannte ich bei einigen Exemplaren einen zottenähnlichen, wenig über 1 μ hohen Besatz."

The endoplasm has a coarsely granulated structure. The nuclei are round or oblong, in size varying from 1 to 4 μ . They are scattered in the endoplasm, unlike the nuclei observed previously by Thélohan, Schröder, etc., who found them situated rather in the middle portion of the endoplasm.

In some young specimens, where the spore formation had begun to take place, I noticed that the nuclei and the pansporoblasts took a peripheral position, while in the middle portion a large round granulated body of distinct contour, but with no nucleus in it, was observed. I could not determine whether it is an accumulation of the endoplasm or an inclusion.

In the older cyst, which is oval-shaped and of about 190 μ in maximum diameter, the ectoplasm becomes thinner than in the younger form. In the periphery of the endoplasm, numbers of nuclei are to be found, and towards the middle portion of it matured spores and several developmental phases of pansporoblasts to spores.

SPORE FORMATION

The nuclei in the plasmodium may be distinguished as vegetative and generative. The latter are always found in a round cell which takes stains more deeply than the surrounding endoplasm. The uninu-

cleate cells are the "sphères primitives" of Thélohan (1895), "pansporoblasts" of Gurley, or "Propagationszellen" of Keysselitz (1908). The propagative cell is of oblong or spindle shape, though usually round in form, with dimensions of 4 to 8 μ . The nucleus is often situated excentrically (Fig. 4). A caryosom, as Keysselitz mentioned, is always found in it. The propagative cell multiplies by division into two or three daughter cells (Figs. 5 to 16). These points correspond to some extent with those of *Myxobolus pfeifferi* according to Keysselitz (1908) and to Mercier, and *Myxidium bergense* according to Auerbach (1912). The nuclear division in the propagative cell of *Sphaeromyxa sabrazesi* according to Schröder (1907 and 1910), *Myxobolus pfeifferi* according to Keysselitz and to Mercier, and *Henneguya psorospermica* according to Auerbach, is reported to be mitotic. In the present form I also observed mitotic division. The chromatin, through the coil stage (Figs. 5 to 7), divides into two parts, exhibiting very often the central spindle (Figs. 8 to 10). In this respect it resembles that of *Myxidium bergense* studied by Auerbach (1912).

The propagative cells resulting from the multiplication go on to spore formation. The greater propagative cell (macrogamete) and the smaller one (microgamete) take an elongated form and associate with their lateral surfaces. At first a space is seen between them (Figs. 17 and 18), and finally the cytoplasm of both cells fuses at the place of contact (Figs. 19 to 23).

The association of two binucleate cells, observed by Schröder in *Sphaeromyxa sabrazesi* and by Keysselitz (1908) in *Myxobolus pfeifferi*, does not exist in the present parasite. The association of the two uninculeate propagative cells in the present Myxosporidia strikingly resembles those observed by Mercier (1904) in *Myxobolus pfeifferi* and by Auerbach (1912) in *Myxidium bergense*. But the nuclei of the associated form do not fuse into one, as Mercier thought happened in *Myxobolus pfeifferi*.

The nuclear change in the pansporoblast coincides to some extent with that mentioned by Auerbach (1912) in *Myxidium bergense*. Instead of uniting into one, the nuclei in the associated form undergo division. The smaller nucleus divides into two at the peripheral position of the pansporoblast, being destined for the nuclei of the pansporoblast (Figs. 23 to 26). The greater nucleus repeatedly divides mitotically with the growth of the pansporoblast (Figs. 22, 25 to 31). In the fully developed pansporoblast, ten nuclei are observed, besides two nuclei of the pansporoblast and the reducing nuclei. At this stage, the contents of the pansporoblast separate into two sporoblasts, each of which contains five nuclei (Fig. 31). Of the five, two are found in a plasmic mass (sporoplasm in the later stage), one is in a cell which usually has a vacuole in it (nucleus for polar capsule and polar fila-

ment), and the remaining two are for the spore membrane. They are clearly recognizable in young spores, as is shown in Figures 32 to 37. When the spore is fully developed, the membrane of the pansporoblast is broken up, and the spores consequently become free in the endoplasm as in bicapsulated *Myxobolus* according to Keysselitz. As I mentioned above, we always recognize several developmental stages of the spore in the older cyst.

MORPHOLOGY OF THE SPORE

The spore has a pyriform shape, with a peculiar attenuated anterior and broadly rounded posterior extremity (Figs. 38 to 45). It has no bilateral symmetry. The spore-membranes of *lateral surfaces* are usually curved in opposite directions (Figs. 39 and 40). The form agrees well with that of *Myxobolus piriformis* described and illustrated by Balbiani and by Thélohan (1895). Spores of the calabash shape, however, occur not infrequently in the present case (Fig. 38). The spore-wall is comparatively thin and composed of two valves, superior and inferior. At the plane of junction the shell is somewhat thickened (Figs. 41 and 44). The surface of the spore usually represents no special structure. Very rarely a single, short, tail-like process about 1.5μ in length is seen at the middle part of the posterior end (Fig. 41). Thélohan (1895) observed a similar abnormality of the spore in *Myxosoma dujardini* and described that "quelques spores anormales ont un prolongement caudal." I also regard the above-mentioned process in certain spores as an abnormality. The length of the spore is about 15μ , the breadth 7 to 8μ and the thickness 5 to 6μ . Thélohan gives the size of the spore of *Myxobolus piriformis* to be 16 to 18 by 7 to 8μ . In the fresh preparations one pyriform polar capsule is observed at the anterior half portion of the spore (Figs. 42 to 44), its dimensions being 7 to 8 by 3 to 4μ . The wall is drawn out anteriorly into a minute duct which pierces the shell near its anterior extremity, affording exit for the polar filament. Thélohan did not measure the size of the polar capsule of *Myxobolus piriformis*. But it seems to be much smaller than the present form (compare his Figures 116 and 117, Plate IX, 1895, with my Figures 38 to 45). Auerbach (1909) observes spores with two polar capsules among unicapsulated spores of *Myxobolus fuhrmanni*. In the present case, all spores have only one large polar capsule each, of which I will speak again when I come to the permanent preparations. Moreover, in some spores, the nucleus of the polar capsule is seen to be attached to it (Figs. 42 and 43). The polar filament is easily extruded from the anterior end of the polar capsule when the spore is treated (Fig. 45) with a reagent like caustic potash, or hydroxyl, or pressed mechanically between the cover and slide glasses. The length of the filament measured after the spore has been

freshly prepared and pressed agrees usually with the measurements of stained ones prepared according to my method (1913). The length of the polar filament of the parasite is 40 to 45 μ , so it is 10 to 15 μ longer than that of *Myxobolus piriformis* measured by Thélohan. The posterior half portion of the spore is filled with sporoplasm. In fresh preparations, it is of a transparent, somewhat granular structure. Treated with iodine-alcohol, there appears a large vacuole stained brownish yellow in the sporoplasm.

In fixed preparations, the anterior end of the spore-membrane is stained very faintly (Figs. 38 to 41). The duct of the polar capsule becomes easily visible. In some spores, close to the anterior end of the polar capsule, there is an oblong mass of protoplasm (Figs. 38 to 40). I took this structure at first to be a polar capsule and compared it with the "Körperchen" of *Pimelodus blochii* of Müller, i. e., *Myxobolus inequalis* described by Gurley. But no such structure is observed in my present preparations, so that I cannot determine whether it is a degenerating polar capsule or some other structure. The nucleus of the polar capsule is always observed in young spores, well stained at the peripheral part of the capsulogenous cell (Figs. 33 to 37). In the sporoplasm, a large iodophile vacuole remains unstained, its diameter being about 3 μ . The iodophile vacuole of *Myxobolus piriformis* observed by Thélohan is smaller than the present one. Two nuclei are always found in the sporoplasm situated closely to each other. They are usually of equal size (Figs. 37 to 41), but sometimes of different dimensions (Fig. 37). The position of the nuclei in the sporoplasm is not always the same. They are seen between the polar capsule and the vacuole (Fig. 39), on a lateral aspect of the vacuole (Fig. 38), or near to the posterior end of the spore (Figs. 40 and 41).

To what genus and what species does the present parasite belong? Because of the presence of an iodophile vacuole, it is clear that it belongs to the genus *Myxobolus*. So far as I am aware, the unicapsulated Myxosporidia known up to the present time are four in number, all belonging to the genus *Myxobolus*:

Myxobolus piriformis Thélohan

Myxobolus unicapsulatus Gurley

Myxobolus fuhrmanni Auerbach

Myxobolus oculi-leucisci Trojan

Of these, *Myxobolus unicapsulatus* is quite different from the present form. If one compares Figures 5, Plate 16 of Müller (1841) with my Figures 38 to 41, one sees great differences between the spores. Moreover, the habitat is quite different. *Myxobolus fuhrmanni* as stated by Auerbach (1909) was found in the connective tissue of the mouth of *Leuciscus rutilus* L. The spore is much larger than the present one

EXPLANATION OF PLATES

All figures except Nos. 1 and 2 are drawn with Abbe's drawing camera.
Figs. 1 to 41 from sections.

Figs. 42 to 45 from fresh preparations.

Staining: Figs. 7, 9, 14, 17, 18, 21, 22 and 35: Giemsa's solution.

All the others: Heidenhain's iron hematoxylin and eosin.

PLATES I AND II

Figs. 1 and 2.—Parts of longitudinal sections of infected branchial lamellae, showing the seat of the parasite. 1, $\times 160$; 2, $\times 320$.

Fig. 3.—A peripheral portion of the parasite, showing the differentiation of the protoplasm. $\times 1000$.

Fig. 4.—A propagative cell from the plasmodium. $\times 2250$.

Figs. 5 to 16.—Division of the propagative cell. $\times 2250$.

Figs. 17 to 21.—Association of the macro- and microgametes. $\times 2250$.

Fig. 22.—Nuclear division of a macrogamete. $\times 2250$.

Figs. 23 and 24.—The same of the microgamete. $\times 2250$.

Figs. 25 to 30.—Several developmental stages of the pansporoblast. $\times 2250$.

Figs. 31 and 32.—Segmentation of the pansporoblast into two sporoblasts. $\times 2250$.

Figs. 33 to 37.—Young spores in development. $\times 2250$.

Figs. 38 to 41. Matured spores. $\times 2250$.

Figs. 42 to 45.—Spores from fresh preparations. $\times 1000$.

Fig. 45.—A spore with polar filament extruded. $\times 1000$.

PLATE I

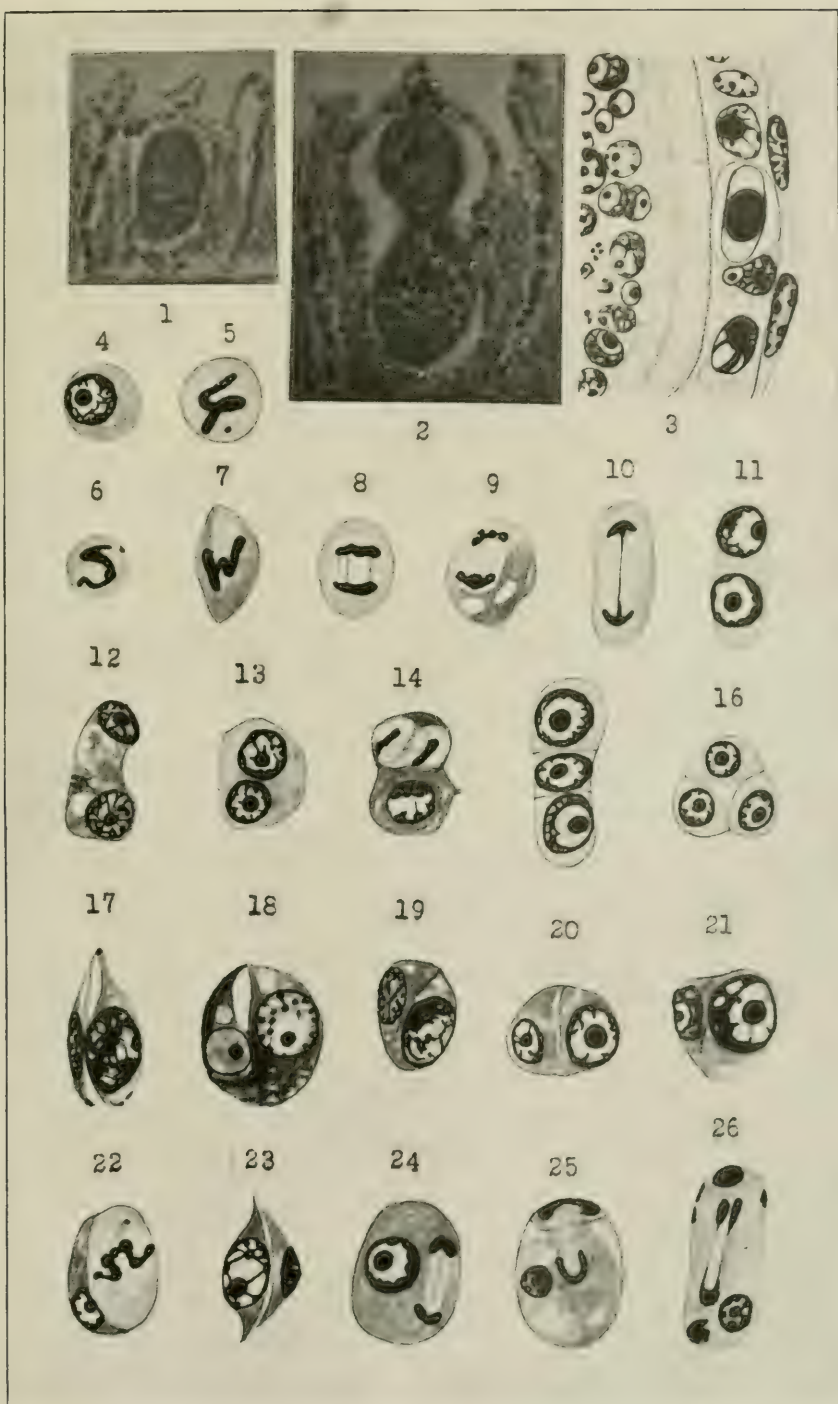
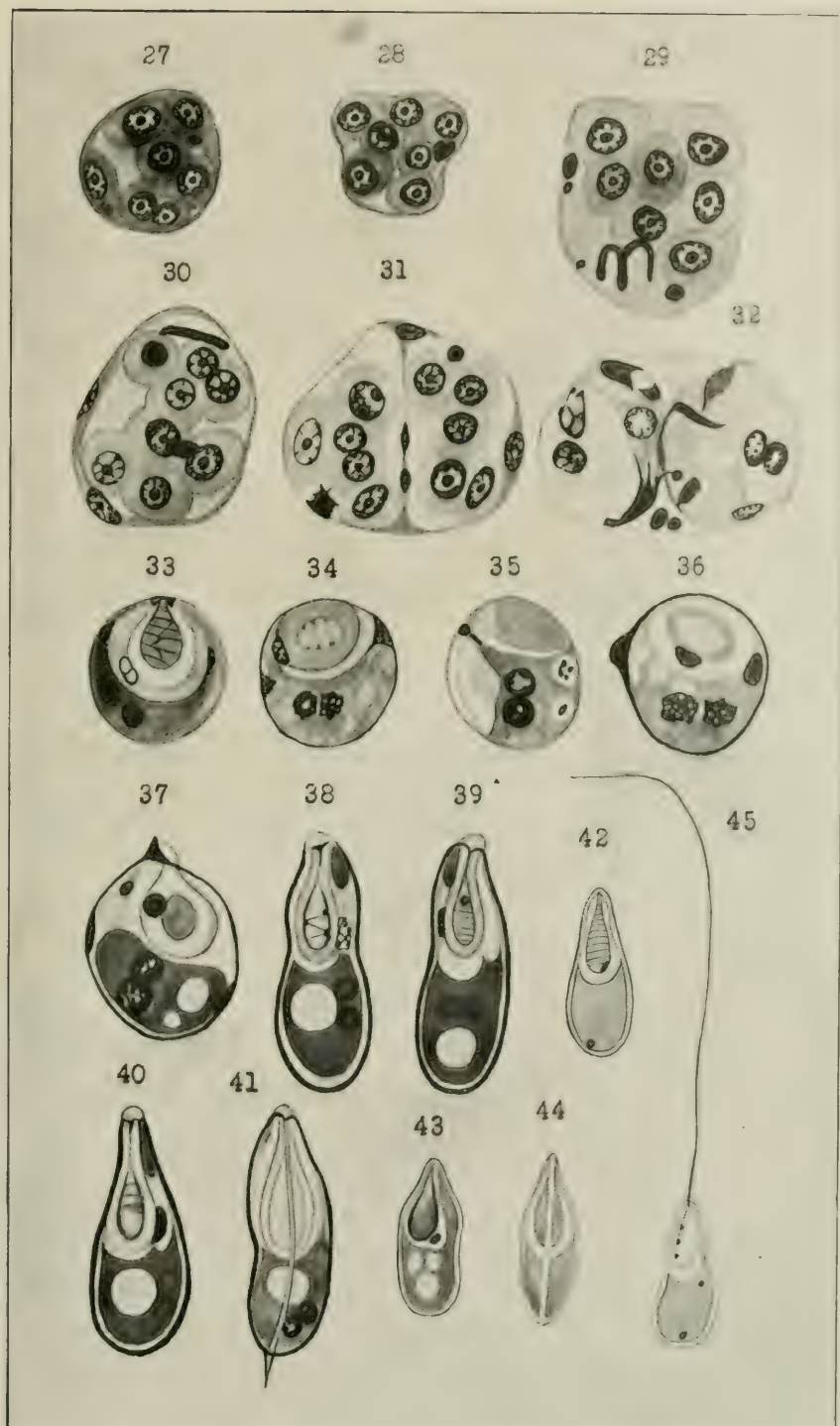


PLATE II



(length 18 to 20 μ ; breadth, about 8 μ ; thickness, 6 μ , and the length of the polar capsule, 9 to 10 μ). The spore membrane is thickened at the posterior end and has 4 to 6 notches. None of these points agree with the observations mentioned above on the present *Myxobolus*. The same is true of *Myxobolus oculi-leucisci*, which was found according to Trojan (1909) in the vitreous humor of the eye of *Leuciscus rutilus* L. Though the size of the cyst is almost equal to my parasite, the spore is smaller and different in structure.

I have spoken only partially of the comparison between the present *Myxobolus* and *Myxobolus piriformis*, and will compare them here again in the following synopsis:

<i>Myxobolus piriformis</i>			The present <i>Myxobolus</i>		
Habitat.....	Branchiae and spleen of <i>Tinca tinca</i> L.; kidney of <i>Misgurnus fossilis</i>		Branchiae of <i>Cyprinus carpio</i> L.		
Cyst.....	"Les kystes branchiaux de cette espèce se reconnaissent à leur minceur: il forment de petites stries filiformes et non des tumeurs sphériques comme le <i>M. ellipsoïdes</i> " (Thélohan, 1895: 348)		Small round cyst in the connective tissue of the gill-filament		
Spore:					
Form.....	Pyriform, with attenuated anterior extremity		Pyriform, with attenuated anterior end; often calabash form		
Size.....	Length	Breadth (Max.)	Length	Breadth	Thickness
	16 to 18 μ	7 to 8 μ	15 μ	7 to 8 μ	5 to 6 μ
Polar.....	Undescribed, figured only, capsule seems smaller		7 to 8 μ by 3 to 4 μ		
Iodine vacuole....	Smaller		About 3 to 4 μ in diameter		

As will be seen from the above comparison, there are great differences in the form of the cyst, the host, the size of the polar capsule, and the length of the polar filament, though the form and dimensions of the spore resemble each other.

Hence I think the *Myxobolus* found by me is a new species, and propose to call it *Myxobolus toyamai* nov. spec. in honor of Prof. Dr. K. Toyama, who kindly introduced me to this branch of protozoology in the year 1909.

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Note. This paper was printed in Japanese in 1915 and is reprinted here at the request of the author.

THE OCCURRENCE OF *BOTHIRIOCEPHALUS LIGULOIDES*
LEUCKART, WITH ESPECIAL REFERENCE
TO ITS DEVELOPMENT

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This larval cestode was first discovered by P. Manson at the post-mortem examination of a Chinaman at Amoy in 1882, and was described as *Ligula mansonii* by Cobbold in the following year. Since then about fifty-five cases have been reported, mostly from Japan with the exception of a few cases from Africa and the Malay Archipelago. The cases were all reported from the human host and it has been questioned for a long time whether this cestode larva was not confined to the human host. Some authors have suspected the existence of this worm in other animals without having actually proved its occurrence outside of man. A very few writers have described unsatisfactorily and uncertainly the occurrence of the cestode larva in question in animals. For instance, Dr. H. Miyake found twelve specimens of a cestode larva in the muscles of a monkey which had recently died, and he reported his belief that they were the same species as the liguloid larva from the human host, comparing them with the specimens and descriptions of previous authors. But the lack of a precise description for his own specimens prevented their positive identification. Other authors also, viz., A. Hirohata and J. Maejima, have proved experimentally that the larva is able to live and grow in the body of the rabbit by transplanting small pieces of the worm with a scolex into the body cavity of that host.

In 1915, during my animal experiments with the encysted larvae of the lung distome, I accidentally came on August 11 across thirty-six specimens of this larval cestode in the body cavity and body wall of the cat employed in my experiment, which died in an extremely anemic and undernourished condition. Some worms were enclosed by a thin fibrous membrane, while others were lying free in the body cavity or in various tissues of the host. One, two, or even three worms were found in one capsule, and the latter were generally smaller in size than the former. The capsules lay in the muscular or subcutaneous tissues, varying in size and shape. A particularly large number of worms were found in the abdominal and pleural muscular wall, where they were tangled together into a ball or were creeping about here and there.

Some portions of the body wall occupied by the worms had suppurated. This agrees with the suppurating condition which is often

reported by various authors for human patients suffering from this cestode larva. Some worms were wound and twisted through various parts of body in such a manner that one end lay in the abdominal cavity and the other end in the abdominal wall, whereas the median portion of the worm lay irregularly in the body cavity and body wall. This state in the body of the host obviously proves the migratory tendency already frequently observed in the human host.

Fresh specimens were extremely mobile, especially in warm physiological salt solution, varying actively the shape and size of body. Large specimens measured 40 to 75 cm. in length and 17 to 20 mm. in breadth. There were also many other specimens in various stages of development or growth. Even in the same individual the length and breadth varies considerably according to the state of contraction. Generally, when it was killed by a fixing agent such as a hot saturate solution of corrosive sublimate, the worm contracted to two-thirds the length of living specimens.

From my own observation of the morphological character and anatomical structure of this worm, as well as its identification by Prof. Dr. Ijima, who was the first writer to describe this cestode larva in Japan, it is evident that the worm in question is the same as Manson's larval cestode of man. Thus I have proved the actual occurrence of this cestode larva in an animal. Furthermore, I am inclined to believe that the normal intermediate host of this tapeworm should not be sought in a human being, but in another animal, though the parasite has not been found previously in the latter host while it has been found so often in the former. Why it seems to occur so often in the human body and so seldom in other animals doubtless depends upon the fact that human parasites are sought more carefully and are hence more frequently found than those of animals. It is obvious that the further development of this cestode larva would be impossible, or less likely at least, if the larvae were normally confined to the human body as a natural intermediate host. I am of the opinion that many animals, domesticated and wild, will be discovered to act as the intermediate host of this larval cestode.

About six months after my discovery, M. Sugimoto in Formosa reported cestode larvae from the pig as *Both. liguloides*, and added that his specimens were quite similar to and probably identical with the specimens of *Sparganum railletii* Rátz 1913 from the pig. The descriptions of Rátz's and Sugimoto's specimens indicate their likeness. But it is doubtful whether they are identical with Manson's larval tapeworm from man or my specimen from the cat.

As stated above, the great majority of the cases infected with this cestode larva have been reported from Japan. They were found in various districts throughout the country, but especially often near

Osaka, the section where thirty-three out of fifty-five cases (60 per cent) are recorded. Thus I am now in a favorable locality to study the worm in question. Many difficulties, however, are encountered in studying the worm, because it occurs very rarely and consequently is found only accidentally in the human body or in other animals. In spite of efforts by various authors at various times, nothing is known of the life-history of the parasite.

I tried twice animal experiments to determine the final host of this larval cestode. In the first case I used two young cats as hosts and the cestode larva from the cat mentioned above. On August 11, 1915, two larvae were fed to one young cat and one larva to the other. The cats unfortunately died on the 17th of the same month from some unknown cause, and on dissection, no parasites of any kind were found in the alimentary tract.

In the second case I made the experiment with the cooperation of my colleague S. Yamada. On June 26, 1916, a specimen of this cestode larva was obtained from a patient who suffered from the worm in the left side of her abdominal wall. The specimen measured 15 cm. in length and 5 mm. in breadth, and was enclosed in a capsule. This larva was given through a catheter to a young dog which was 27 days old, and had been reared in our anatomical department. Before the feeding, the feces of the dog were repeatedly examined for parasite eggs, and we found the eggs of *Dipylidium caninum* only. Afterwards we examined daily for parasite eggs; and on the thirteenth day after experimental feeding we first found a new kind of parasite egg which increased in numbers day after day, and ultimately attained a maximum condition that continued until the animal was killed.

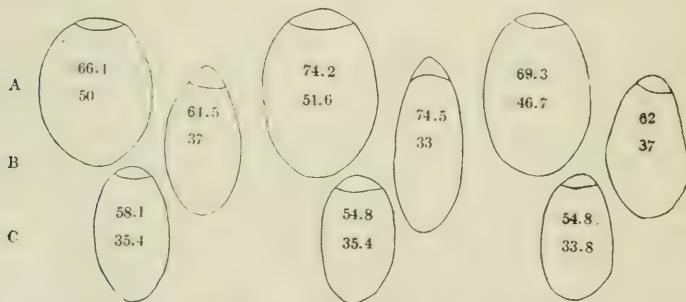
The eggs are elongated oval in shape, tapering toward both poles, markedly sharper at the anterior than at the posterior end. On the anterior pole they are provided with a small operculum. They are mostly symmetrical, the curvature on the two sides of the long axis being unequal. One may find a minute globular thickening of the egg-shell at the posterior pole in some eggs, such as is observed in the eggs of certain distomes. The eggs closely resemble those of *Dibothriocephalus*, but are darker brown in color and different in shape. Some measurements in microns are as follows:

	1	2	3	4	5	6	7	8	9
Length	76.9	75.8	74.5	69.3	68.7	68.5	64.5	64.5	62
Breadth	43.5	37	33	37.1	38.7	36.3	41.9	37	37
Ratio	1.76:1	2.04:1	2.25:1	1.86:1	1.77:1	1.88:1	1.53:1	1.74:1	1.67:1

On August 26, over two months after the feeding, we killed the dog and examined it for the parasites. We found a few specimens of *Ancylostomum*, two of *Dipylidium caninum*, and one large tapeworm

belonging to the genus *Dibothriocephalus*. This measured 2.5 m. in length and 12 mm. in maximum breadth, the maximum length of proglottis being 2 mm. When alive it was very active and its length and breadth varied considerably, as is usual among cestodes. The posterior extremity showed a bifurcated anomaly, the body being divided into two halves near the median line, one half being 80 mm. long by 5 mm. broad, and the other half 50 mm. long by 3 mm. broad. The length of a proglottis in the bifurcated portion was constant (2 mm.).

From observations on the external features and the internal structure we easily identified this specimen as belonging to the genus *Dibothriocephalus*, and bearing a close resemblance to *Dib. latus*. But I doubt whether the worm is identical with *Dib. latus* of the dog previously reported. The known species of the genus *Dibothriocephalus* from the dog are *Dib. fuscus* Krabbe 1886, *Dib. serratus* (Diesing,



Eggs of dibothriocephaloid cestodes from the various hosts. $\times 620$.

A. From human host. B. From our dog used in experiment. C. From lion.

1850), *Dib. cordatus* Leuck. 1863, and *Dib. latus* (L. 1748). The worm in question may easily be distinguished from any of the first three species mentioned above.

I will add a few words on the comparison of this worm with *Dib. latus* which it resembles in some characteristics and not in others. Resemblance exists in respect to scolex form (though not accurately observed on account of irregular distortion by contraction), general form of the strobila, proportion of length and breadth of the proglottis in every part of the strobila, and general structure of internal organs and tissues. A remarkable point of difference is in the shape of the eggs. The eggs of this worm are easily distinguished from those of *Dib. latus* by their shape and the ratio of length to breadth.

The eggs of the new worm are elongated oval in shape and mostly asymmetrical, the curvature on both sides of long axis being unequal, and they taper toward both poles, ending slightly pointed. The anterior

pole is more pointed than the posterior, as stated above. The proportion of length to breadth, varying from 1.53:1 to 2.25:1, is greater than that (1.16:1 to 1.48:1) of *Dib. latus*. The eggs of *Dib. latus* are oval, both poles ending equally rounded and relatively broader than those of the new worm.

Measurements show that there is a great variation of egg size in *Dib. latus* according to the species of the host, whether human or other animal. The eggs of *Dib. latus* from other animals are the same in shape but much smaller than those from the human host. The next table serves to show the variation of egg-size.

	Human 1	Human 2	Human 3	Lion 1	Lion 2	Lion 3
Length	74.2	69.3	66.1	58.1	54.8	54.8
Breadth	51.6	46.7	50	35.4	35.4	33.8
Ratio	1.16:1	1.48:1	1.32:1	1.62:1	1.54:1	1.62:1

From the above tables it is evident that the eggs of the new worm are midway in size between those of the tapeworms from the human host and from the lion.

In spite of such a great difference in the size of eggs, dibothriocephaloid cestodes from human host and other animals have been considered to be the same species as *Dib. latus* by all previous authors. If this identification by the previous authors is unquestionable, the new worm might be identified as *Dib. latus* and the remarkable difference in size and shape of eggs be considered a mere variation among the same species. If this supposition is right, one must reconsider the animal experiment. If the worm obtained from the dog under experimentation is supposed to be *Dib. latus*, the dog must have swallowed a larva of this cestode species; that is, have eaten the raw meat of salmon trout, which is considered in Japan to be the only species of fish harboring the larval form of this cestode. During our experiment the dog was always kept in a cage and fed regularly, so that he never obtained fish as food or accidentally. Before the experiment the dog was nursed by the mother or fed upon remnants of food which were generally boiled or roasted and could not be expected to contain a living larval cestode. Such a careful feeding experiment makes it impossible to think of an accidental infection with *Dib. latus* by food containing the larva. Therefore I am doubtful that the worm is surely identical with *Dib. latus*, and consequently it is a question whether the dibothriocephaloid cestodes from the human host and from other animals have been correctly identified by previous authors.

There is only one remarkable character, the egg size and shape, useful to distinguish positively the worm from the known species, *Dib. latus*. Egg size and shape of parasites, however, is generally assumed

to play an important rôle in determining species. Agreeing with this point of view supported by H. B. Ward, A. Looss, and other helminthologists, I have the following opinion in respect to the eggs: I am inclined to believe the worm in question will be experimentally determined hereafter to be the matured form of Manson's larval cestode and quite different from *Dib. latus*. Consequently, some cases — especially from natural infection — of supposed *Dib. latus* from other animals such as the lion, dog, cat, etc., previously reported might have been mistakenly identified and really might have been the mature form of Manson's larval cestode, or indeed of still another species.

The natural mode of infection by *Dib. latus* also seems to support my supposition. To become infected with *Dib. latus*, it is necessary to eat raw fresh meat of fish harboring a larval form of this tapeworm; such fish are the salmon trout in Japan, or pike, salmon, perch, etc., in Europe. Generally the dog is fond of uncooked meat, but not of fish, in Japan at least; so it is unnatural and very rare for dogs to get fresh fish as food. Therefore it is puzzling to me why dogs are infected so often with this tapeworm in Japan, especially in the districts where the fish intermediate host is not found. The same difficulty holds good for the case of the lion, tiger, and other animals which are frequently infected with *Dib. latus*, although these wild beasts are accustomed to eat other weaker beasts and birds, but not fish so far as we know.

It has already been proved, however, by previous authors that the plerocercoid larva of *Dib. latus* from fish can develop to the adult form in the alimentary tract of dog and cat. So it is probable, I think, that the dog and perhaps other beasts like the lion, tiger, etc., can become infected with two kinds of dibothriocephaloid tapeworms, viz., the well-known species *Dib. latus*, and a new species, the adult form of Manson's liguloid larva.

At any rate to have found the adult form of Manson's larval tapeworm is both important and interesting, not only as a contribution to the knowledge of the development of the worm itself, but for the determination of species of dibothriocephaloid cestodes from the human host and from animals.

In closing, I wish to express my appreciation to Prof. Dr. Ijima, Chief of the Zoological Institute, Tokyo Imperial University, for his kind identification of the cestode larvae, and to Prof. Dr. Sakurane, Chief of the Dermatological Department of our hospital, for his courtesy in placing the material at my disposal.

A FURTHER NOTE ON THE LIFE-HISTORY OF
*GONGYLONEMA SCUTATUM**

BRAYTON H. RANSOM AND MAURICE C. HALL

In two recent papers, one of which is an admirable monograph of the larval forms of the heteroxenous parasitic nematodes and the other a comprehensive study of the Gongyloneiminae of North Africa, Seurat (1916: 739; 1916a: 358) has expressed the opinion that certain larval nematodes found in various species of coprophagous beetles (*Aphodius*, *Onthophagus*) which we identified (Ransom and Hall, 1915: 154; 1916: 80-86) as the larvae of *Gongylonema scutatum* probably belong to another species, *G. mucronatum* Seurat 1916. The adults of the latter species have been found by Seurat in the mucosa of the esophagus and base of the tongue of the Algerian hedgehog (*Erinaceus algirus* Duv.). It has not yet been recorded from sheep or cattle. Easily recognizable differences between *G. scutatum* and *G. mucronatum* are as follows, the statements relative to structural characters of the latter being taken from Seurat's description:

In *G. scutatum* the cervical papillae are situated about midway between the anterior border of the nerve ring and the anterior end of the body, each in the center of a rounded cuticular shield; in *G. mucronatum* they are situated at the anterior third of the distance between the anterior border of the nerve ring and the anterior end of the body and are not inserted in the center of a cuticular shield. In *G. scutatum* the caudal pores are subterminal, in *G. mucronatum* situated at about two-thirds of the distance between the anus and the tip of the tail. In *G. scutatum* there is no papilla in the neighborhood of the vulva; in *G. mucronatum* an unpaired papilla is situated on the ventral surface of the body about 0.1 mm. behind the vulva.

In view of Seurat's opinion we have examined in detail numerous specimens of *Gongylonema* collected from the esophagus of sheep and cattle in various parts of the United States and have failed to find among them any corresponding to *G. mucronatum*, or to any species other than *G. scutatum*. If *G. mucronatum* is present in the United States, it is not likely that it occurs in sheep or cattle; at least, it must be rare in these hosts. Consequently, even considering the fact that we did not make a detailed microscopic examination of every worm from which eggs were obtained for feeding to insects in the experiments recorded in our former paper, but depended in many instances

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upon the gross appearance of the parasites as sufficient for their identification, it seems scarcely possible that there should have been invariably present in the material fed to the insects not only the eggs of *G. scutatum*, but also those of another species, whose presence in the sheep or cattle from which our material was obtained we constantly overlooked. The only apparent possibilities of error affecting our interpretations of the results of our experiments in feeding croton bugs and beetles in addition to the one just mentioned are (1) that the insects were already infested, and (2) that during the progress of the experiments they acquired the parasites from some other source than the material originally fed to them. In the case of the beetles we realized that some of them probably already harbored the parasites at the beginning of the experiments and gave due consideration to this probability in interpreting our observations; but the possibility of such a circumstance in the case of the croton bugs is very slight in view of the fact that we have frequently examined croton bugs caught from the same places as those used in the experiments without finding *Gongylonema* larvae. The second possibility is also very slight in the case of the croton bugs, as they were kept during the experiments either without access to food or fed only on bread crumbs or similar food unlikely to contain nematode eggs. Furthermore, croton bugs kept in a similar manner and used in other experiments have never shown *Gongylonema* larvae. The beetles used in our experiments in some instances were kept in containers with unsterilized feces from sheep and consequently might have acquired their parasites from this source, but in certain instances the feces in which the beetles were kept and upon which they fed were sterilized. In the latter case, even with beetles already infested, one would be justified in considering as we did that the newly hatched *Gongylonema* embryos observed in large numbers a day or two after feeding, and the developing larvae in progressive stages observed later in due course of time, came from the eggs contained in the material fed to the beetles. So far as we are able to perceive after reviewing our records and recollections, our experiments in the feeding of *Gongylonema* eggs to insects were adequately safeguarded and controlled in all essential respects, with the exception that possibly sufficient care was not taken to exclude the eggs of species other than *G. scutatum*. With this possibility of error in view, slight though it is, the senior author has carried out a new series of experiments in feeding croton bugs.

The insects used in the recent experiments were kept in flasks closed by cotton plugs. In addition to being supplied with material containing the *Gongylonema* eggs they were occasionally given fresh bread crumbs and a few drops of water. Numerous individuals caught from time to time in the same place as those used in the experiments

were examined and found to be free from infestation. Some were also kept in flasks and fed bread and water, but no *Gongylonema* eggs, as controls against those fed *Gongylonema* eggs. The controls remained free from infestation. The *Gongylonema* material for feeding was obtained from a few infested gullets of sheep and cattle procured at an abattoir. All of the parasites were carefully removed from the gullets. By microscopic examination the fact was established that all of the worms present in each of the gullets corresponded to *G. scutatum*, special attention being given to the cervical papillae, absence of a postvulvar papilla, and subterminal location of the caudal pores. Female worms thus obtained and identified were washed in several changes of physiological salt solution to reduce the chances of foreign eggs adhering to their bodies, cut into small pieces, mixed with bread crumbs, and placed in the flasks containing the captive croton bugs. As in our former experiments, these croton bugs became infested with the larvae of *Gongylonema*. Individuals were examined at intervals, and various stages ranging from the newly hatched larvae up to the encysted forms, and exhibiting the same characteristics of structure as those described in our former paper, were recovered. An encysted larva taken from a croton bug seven weeks after eggs of *Gongylonema scutatum* were placed in the flask in which it was kept, measured 1.9 mm. in length by 0.06 mm. in diameter. The pharynx was 0.035 mm. in length, the esophagus 1.2 mm., its muscular portion 0.23 mm. The cervical papillae were 0.07 mm. from the anterior end of the body, the nerve ring 0.125 mm., excretory pore 0.21 mm. The anus was 0.09 mm. from the tip of the tail, the caudal pores 0.025 mm.

With reference to our experiments in feeding sheep with infested beetles and croton bugs (Ransom and Hall, 1916) it may be noted that their results if considered by themselves were less conclusive than those of the experiments in feeding *Gongylonema* eggs to insects, because of the small number of animals used and the lack of complete control of all the conditions which might have affected the experiments. Furthermore, no attempt was made to obtain a series of steps in the development in sheep between the larva and the adult. As a matter of fact in our former paper we did not insist upon the conclusiveness of the sheep-feeding experiments and considered them of importance only when viewed in the light of other evidence without which they would have been much less significant. Even though the experimental evidence that the larvae of *Gongylonema scutatum* in dung beetles develop to maturity in sheep and other suitable mammalian hosts when the insects are ingested by these animals is less complete than that as to the development of the larval stage in the insects, such evidence as we have is in exact accord with that hypothesis, which moreover by analogy is strongly supported by the known facts in the life histories

of other parasites, and we are justified in assuming until very definite evidence to the contrary is brought forward, that sheep, cattle, and other suitable host animals become infested with *Gongylonema scutatum* as a result of swallowing infested insects, under natural conditions probably various species of dung beetles.

From the foregoing it is evident that the validity of the results of our work on the life history of *Gongylonema scutatum* has not been affected by the question raised by Seurat regarding the correctness of our identification of the larval nematodes which we found in coprophagous beetles and under experimental conditions in croton bugs. It is also evident that the nematodes found by Seurat (1916: 739, Fig. 5; 1916a: 315, 346) in several species of Blaps, and because of the subterminal position of the caudal pores considered by him to be the larvae of *G. scutatum*, cannot belong to this species, unless it is a species whose larvae are characterized by an unusual degree of polymorphism. Whether the nematodes occurring in various species of coprophagous beetles in Algeria, which are strikingly similar to those which we have shown to be the larvae of *G. scutatum*, belong to *G. mucronatum* as Seurat (1916a: 317, 346, Fig. 11) supposes, remains to be determined. The basis upon which Seurat identified them as *G. mucronatum* is the location of the caudal pores at a considerable distance from the tip of the tail, a character in which they agree with the adults of this species. Clearly, however, apparent similarities in details of structure are not sufficient in the absence of other evidence to justify definite conclusions as to the specific identity of larval and adult nematodes, and further investigations will be necessary before the larvae described by Seurat as such can be accepted as the larvae of *G. mucronatum*. Because of their close agreement in structure with the larvae of *G. scutatum*, it is quite probable that they actually belong to this species, the adult stage of which Seurat has found to be common in Algeria.

SUMMARY

Despite the doubts raised by Seurat in recent publications, the conclusions expressed in our former papers on the life history of *Gongylonema scutatum* are still valid. It has been definitely proved that dung beetles and croton bugs fed upon the eggs of *G. scutatum* become infested with an encysted larval stage of the parasite, and the evidence is very strong, if not quite conclusive, that sheep, cattle, and other suitable mammalian hosts become infested as a result of swallowing infested insects (usually under natural conditions, various species of dung beetles).

The nematodes found in several species of Blaps in Algeria and identified by Seurat as the larvae of *G. scutatum* belong to some other species.

It is not improbable that the nematodes found in Algerian beetles which Seurat has considered to be the larvae of *G. mucronatum* in reality belong to *G. scutatum*.

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NOTES

The intermediate host of *Schistosoma mansoni* in Venezuela is discussed in a recent important paper by Drs. Juan Iturbe and Eudoro González. By infection experiments with the mollusks of the valley around Caracas, the true intermediate host was found to be *Planorbis guadelupensis*. The miracidia of *S. mansoni* developed into sporocysts within this host, and subsequently furcocercous cercariae were obtained from it. By immersion in water infected with these cercariae and by feeding experiments white mice were brought to develop adult *S. mansoni*. The paper is illustrated with two microphotographic plates.

A recent number of Japanese Medical Literature states that *Schistosoma japonicum* is reported by Narabayashi to depend on a small snail in the rice fields as its intermediate host. According to Pilsbury this snail should properly be called *Blanfordia nosophora* Robson. The cercariae invade the skin even if the latter is only damp. A definite relation exists between schistosomiasis and a skin disease called "kabure."

The same journal reviews a paper on *Paragonimus westermanii* in the Korean Medical Society Journal in which Muneta records from an autopsy the abundant occurrence of the fluke cysts in the abdomen under the peritoneum; liver, spleen, heart, sternum and the cheek were also invaded. Some nodules contained adult worms; others did not.

Davainea formosana, a new human tapeworm from Formosa and Tokyo, is described by Akashi in the Journal of the Formosa Medical Society and abstracted in Japanese Medical Literature. The specimens came from children. The species may be distinguished from the other member of the same genus long known as a human parasite by the following characters:

	<i>Davainea formosana</i>	<i>D. madagascarensis</i>
Length of body.....	43 cm.	25 to 35 cm.
Number of joints.....	Over 700	500 to 700
Hooks on suckers.....	None	Armed
Adult segment.....	2.0 to 2.5 by 1.0 mm.	2.0 by 1.4 mm.
Egg masses.....	300-400	120-150
Size of egg masses.....	0.26 by 0.13 mm.	0.3 mm.
Size of eggs.....	99 by 46 μ	40 μ
Size of onchosphere.....	12 to 14 μ	15 μ

"ECHINORHYNCHUS MONILIFORMIS" IN NORTH AMERICA

My attention has been called to the fact that in the Proceedings of the Philadelphia Academy (1874:76) is recorded with brief comments an exhibit by H. C. Chapman of specimens of *Echinorhynchus moniliformis* from the alimentary canal of the fox squirrel (*Sciurus vulpinus*). Stiles and Hassall also in their Preliminary Catalog of the Parasites, etc., (1894:352) list the species from *Sciurus niger* as found in the Leidy Collection. The statements in my note must be corrected in accordance with these facts. No data are given in these brief records to determine whether the authors mentioned above had before them the true European species or the North American form which I have studied.

H. B. W.

INDEX TO VOLUME III

	PAGE
Animal Parasites of Man, by Fantham, Stevens, and Theobald (review) ..	139
Arthropoda, Observations on Polycystid Gregarines from.....	65
<i>Ascaris triquetra</i> Schrank in Dogs, A Case of the Occurrence of.....	39
<i>Attacus cynthia</i> Drury, Note on a Species of Nosema Infecting.....	136
Book Reviews, see Reviews.	
<i>Bothrioccephalus liguloides</i> Leuckart, The Occurrence of, with Especial Reference to Its Development.....	171
Brookover, Charles: Diptera in the Human Intestine (note).....	141
Case of the Occurrence of <i>Ascaris triquetra</i> Schrank in Dogs.....	39
Cawston, F. G.: The Cercariae of Natal.....	131
Cercariae of Natal.....	131
of the Bitter Root Valley, Montana, Notes on the.....	105
Notes on Two Free-Living Trematodes from North America.....	10
Cestodes from the Spotted Sting-Ray, Notes on Two.....	34
Contributions to the Study of Parasitic Protozoa. II. <i>Myxobolus toyamai</i> nov. spec., a New Myxosporidian Parasite in <i>Cyprinus carpio</i> L.....	163
III. Notes on Myxosporidia Found in Some Fresh-Water Fishes of Japan, with the Description of Three New Species.....	3
Cooper, A. R., see Job, Thesle T.	
Crab, <i>Helice tridens</i> (de Haan), On a Trematode Larva Encysted in a....	76
Crithidia sp.? (note).....	142
<i>Cynomys ludovicianus</i> , <i>Cytolichus penrosei</i> , a New Arachnoid Parasite Found in the Diseased Lungs of a Prairie Dog.....	82
<i>Cyprinus carpio</i> L., <i>Myxobolus toyamai</i> nov spec., a New Myxosporidian Parasite in	163
<i>Cytolichus penrosei</i> , a New Arachnoid Parasite Found in the Diseased Lungs of a Prairie Dog, <i>Cynomys ludovicianus</i>	82
Dauercystformation of <i>Trichomonas intestinalis</i>	28
<i>Dazainca formosana</i> (note).....	182
Development of Gregarines and Their Relation to the Host Tissues: (I) In <i>Stenophora lactaria</i> Watson.....	124
Diptera in the Human Intestine (note).....	141
" <i>Echinorhynchus moniliformis</i> " in North America (note).....	141, 182
Effects of Radiation on the Development of <i>Trichinella spiralis</i> , with Respect to Its Application to the Treatment of Other Parasitic Diseases.....	43
<i>Endamoeba buccalis</i> . I. Its Multiplication and Periodicity.....	143
Faust, Ernest Carroll: Notes on the Cercariae of the Bitter Root Valley, Montana	105
Fishes, Notes on Some Nematodes from Fresh-Water.....	57
Fundulus, Further Observations on <i>Myxobolus musculi</i> from.....	91, 150
Further Note on the Life History of <i>Gongylonema scutatum</i>	177
<i>Gongylonema scutatum</i> , A Further Note on the Life History of.....	177
Gregarines: Development of, and Their Relation to the Host Tissues: (I) In <i>Stenophora lactaria</i> Watson.....	124
from Arthropoda, Observations on Polycystid.....	65

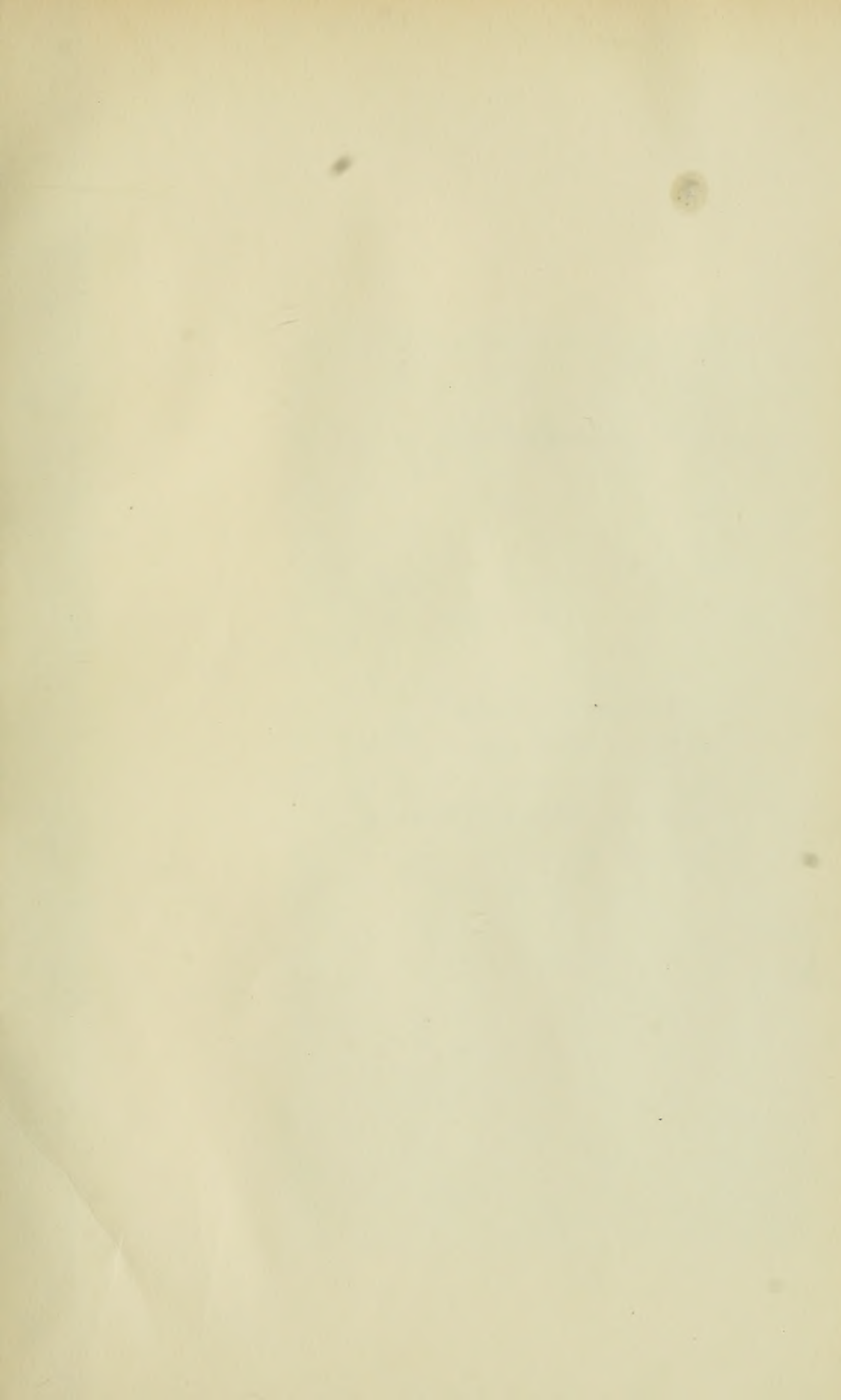
Hahn, C. W.: On the Sporozoon Parasites of the Fishes of Woods Hole and Vicinity. I. Further Observations on <i>Myxobolus musculi</i> from <i>Fundulus</i>	91
II. Additional Observations on <i>Myxobolus musculi</i> of <i>Fundulus</i> and a Nearly Related Species, <i>M. pleuronectidae</i> of <i>Pseudopleuronectes americanus</i>	150
Hall, Maurice C., see Ransom, Brayton, H.	
Harvard School of Tropical Medicine (note)	42
<i>Helice tridens</i> (de Haan), On a Trematode Larva Encysted in a Crab....	76
Honeij, James A.: see Tyzzer, E. E.	
House Fly, Dangerous (note)	142
Ishiwata, Shigetane: Note on a Species of <i>Nosema</i> Infecting <i>Attacus cynthia</i> Drury	136
Japan, Notes on Myxosporidia Found in Some Fresh-Water Fishes of, with the Description of Three New Species	3
Japanese Medical Literature (reviews)	42, 140
Job, Thesle T., and A. R. Cooper: Notes on <i>Porocephalus globicephalus</i>	138
Kamm, Minnie Watson: The Development of Gregarines and Their Relation to the Host Tissues: (I) In <i>Stenophora lactaria</i> Watson	124
See also Watson, Minnie E.	
Kudo, Rokusaburo: Contributions to the Study of Parasitic Protozoa. II. <i>Myxobolus toyamai</i> nov. spec., a New Myxosporidian Parasite in <i>Cyprinus carpio</i> L.	163
III. Notes on Myxosporidia Found in Some Fresh-Water Fishes of Japan, with the Description of Three New Species	3
<i>Leishmania brasiliensis</i> (note)	142
Life History of <i>Gongylonema scutatum</i> , A Further Note on the	177
Linton, Edwin: Notes on Two Cestodes from the Spotted Sting-Ray....	34
Lühe, Max (note)	42
Lynch, Kenneth M.: Dauercystformation of <i>Trichomonas intestinalis</i>	28
Magath, Thomas B., see Ward, Henry B.	
Man, Parasites of:	
Animal Parasites of Man, by Fantham, Stephens, and Theobald (review)	139
Dauercystformation of <i>Trichomonas intestinalis</i>	28
<i>Davainea formosana</i> (note)	182
Diptera in the Human Intestine (note)	141
<i>Endamoeba buccalis</i> , I. Its Multiplication and Periodicity	143
Japanese Medical Literature (review)	42
Medical and Veterinary Entomology, by William B. Herms (review) ..	90
Occurrence of <i>Bothriocephalus liguloides</i> , with Especial Reference to Its Development	171
Medical and Veterinary Entomology, by William B. Herms (review)....	90
Michigan University Biological Station (note)	90
<i>Myxobolus musculi</i> from <i>Fundulus</i> , Further Observations on	91, 150
<i>toyamai</i> nov spec., a New Myxosporidian Parasite in <i>Cyprinus carpio</i> L.	163
Myxosporidia, Notes on, Found in Some Fresh-Water Fishes of Japan, with the Description of Three New Species	3
Nematodes from Fresh-Water Fishes, Notes on Some	57
<i>Nosema</i> Infecting <i>Attacus cynthia</i> Drury, Note on a Species of	136
Note on a Species of <i>Nosema</i> Infecting <i>Attacus cynthia</i> Drury	136
Notes	42, 90, 141, 182

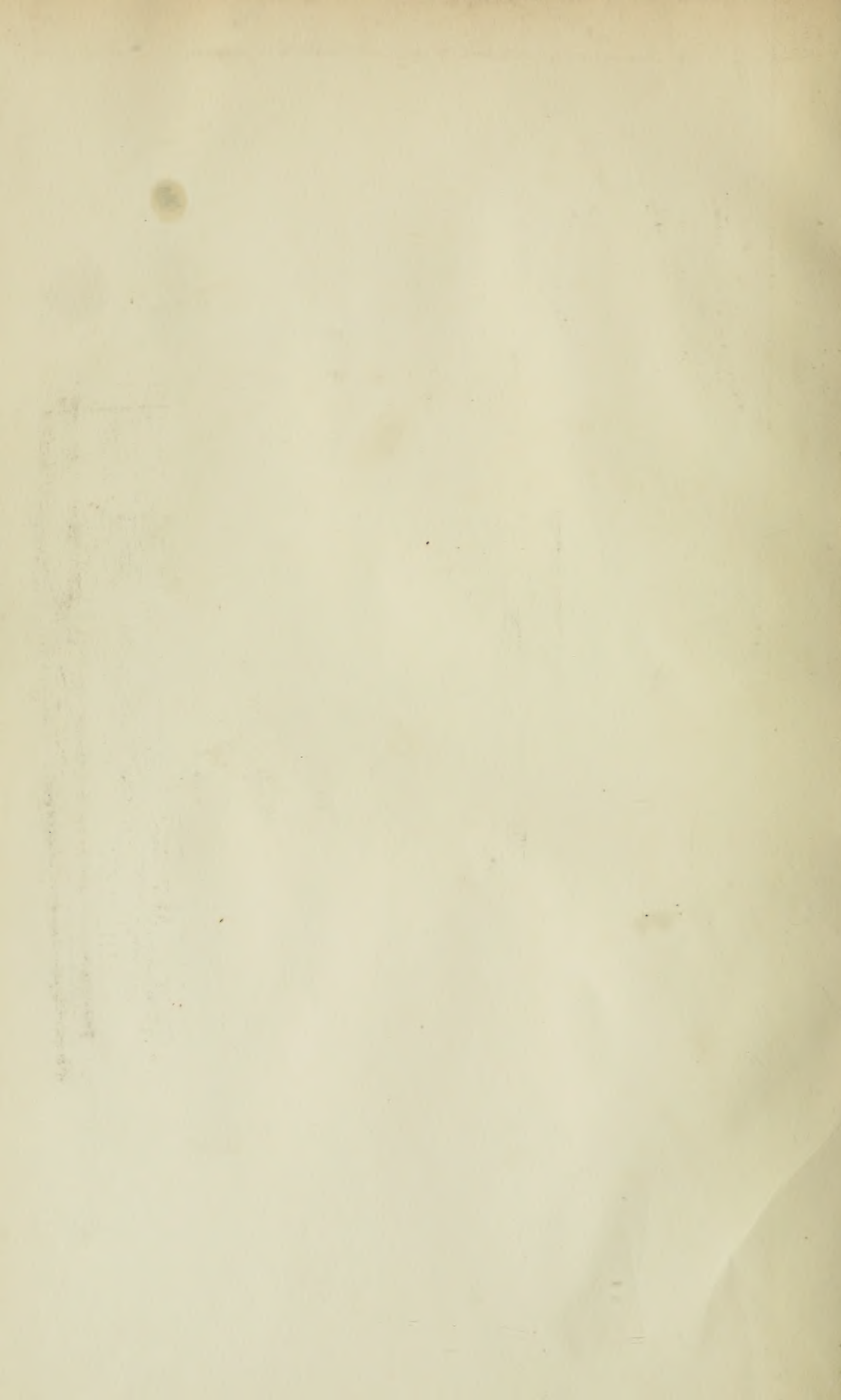
INDEX TO VOLUME III

	185
	PAGE
Notes on <i>Porocephalus globicephalus</i>	138
Some Nematodes from Fresh-Water Fishes.....	57
the Cercariae of the Bitter Root Valley, Montana.....	105
Two Cestodes from the Spotted Sting-Ray.....	34
Two Free-Living Larval Trematodes from North America.....	10
Nowlin, Nadine: <i>Endamoeba buccalis</i> . I. Its Multiplication and Periodicity	143
Observations on Polycystid Gregarines from Arthropoda.....	65
Occurrence of <i>Bothriocephalus liguloides</i> Leuckart, with Especial Refer- ence to Its Development.....	171
On a Trematode Larva Encysted in a Crab, <i>Helice tridens</i> (de Haan)....	76
On the Anatomy and Relationships of Some North American Trematodes	21
On the Sporozoon Parasites of the Fishes of Woods Hole and Vicinity.	
I. Further Observations on <i>Myxobolus musculi</i> from Fundulus.....	91
II. Additional Observations on <i>Myxobolus musculi</i> of Fundulus and a Nearly Related Species, <i>M. pleuronectidae</i> of <i>Pseudopleuronectes</i> <i>americanus</i>	150
<i>Paragonimus westermanii</i> , New Human Tapeworm (note).....	182
<i>Planorbis guadelupensis</i> (note).....	182
<i>Porocephalus globicephalus</i> , Notes on.....	138
Protozoa, Contributions to the Study of. II. <i>Myxobolus toyamai</i> nov spec., a New Myxosporidian Parasite in <i>Cyprinus carpio</i> L.....	163
III. Notes on Myxosporidia Found in Some Fresh-Water Fishes of Japan, with the Description of Three New Species.....	3
Radium Emanations:	
Effects of Radiation on the Development of <i>Trichinella spiralis</i> , with Respect to Its Application to the Treatment of Other Parasitic Diseases	43
Ransom, Brayton H., and Maurice C. Hall: A Further Note on the Life History of <i>Gongylonema scutatum</i>	177
Reviews:	
Animal Parasites of Man, by Fantham, Stephens, and Theobald.....	139
Japanese Medical Literature.....	140
Medical and Veterinary Entomology, by William B. Herms.....	90
<i>Schistosoma japonicum</i> (note).....	182
<i>mansoni</i> (note)	182
<i>Stenophora lactaria</i> Watson, The Development of Gregarines and Their Relation to the Host Tissues: (I) In.....	124
Sting-Ray, Notes on Two Cestodes from the Spotted.....	34
Stunkard, Horace W.: On the Anatomy and Relationships of Some North American Trematodes	21
Trematode Larva Encysted in a Crab, <i>Helice tridens</i> (de Haan). On a....	76
Trematodes from North America, Notes on Two Free-Living Larval....	10
on the Anatomy and Relationships of Some North American.....	21
<i>Trichinella spiralis</i> , The Effects of Radiation on the Development of, with Respect to Its Application to the Treatment of Other Parasitic Diseases	43
<i>Trichomonas intestinalis</i> , Dauercystifformation of.....	28
Tropical Medicine, Harvard School of (note).....	42
Tyzzer, E. E., and James A. Honeij: The Effects of Radiation on the Development of <i>Trichinella spiralis</i> , with Respect to Its Application to the Treatment of Other Parasitic Diseases.....	43
Walton, A. C.: A Case of the Occurrence of <i>Ascaris triquetra</i> Schrank in Dogs	36
Ward, Henry B.: " <i>Echinorhynchus moniliformis</i> " in North America (note and correction)	141, 182
Notes on Two Free-Living Larval Trematodes from North America..	10

	PAGE
Ward, Henry B., and Thomas B. Magath: Notes on Some Nematodes from Fresh-Water Fishes.....	57
Watson, Minnie E.: Observations on Polycystid Gregarines from Arthropoda	65
See also Kamm, Minnie Watson	
Weidman, Fred D.: <i>Cyrtolcichus penrosci</i> , a New Arachnoid Parasite Found in the Diseased Lungs of a Prairie Dog, <i>Cynomys ludovicianus</i>	82
Yoshida, Sadao: Occurrence of <i>Bothrioccephalus liguloides</i> Leuckart, with Especial Reference to Its Development.....	171
On a Trematode Larva Encysted in a Crab, <i>Helice tridens</i> (de Haan)...	76







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